LINKING STEM AND LEAF TRAITS TO LEAF PHENOLOGY IN NATIVE AND NON-NATIVE WOODY PLANTS OF EASTERN DECIDUOUS FORESTS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Maria Suk Smith

January 2014

ABSTRACT

Non-native woody plants pose significant threats to biodiversity, and thus ecosystem goods and services. Many non-natives exhibit suites of traits such as higher productivity, which ties closely to tissue composition and above- and below-ground resource use. A major driver of plant productivity and a strategy of many successful non-natives is extended leaf phenology. Here, we explore both above- and below-ground morphological and physiological traits that potentially enable early and late seasonal leaf productivity. In the first study, we relate belowground seasonal fine root production, biomass, and physiological function through nitrogen (N) uptake to aboveground leaf phenology of two congeneric pairs of native and invasive vines (*Lonicera*) and shrubs (*Rhamnus*). The second study explores the capacity of non-native plants to more efficiently conduct water transport through differences in wood anatomical traits and arrangement of those traits.

BIOGRAPHICAL SKETCH

Maria Smith was born June 1, 1987 in the small coastal town of Poquoson, Virginia, daughter of Dr. George Smith and Hong Suk Yiu, a native Korean. She spent her childhood unknowingly exploring one of the most unique wetland and barrier island ecosystems in the United States. In school, she excelled in math and science, an ardent student of Bill Nye and the Magic School Bus. During grade school, she expressed interest in the fields of neuroscience and medicine, thus an aptitude for biology. A graduate of Poquoson High School, she attended Virginia Commonwealth University (VCU) in the fall of 2005 on a dean's scholarship for biology with a concentration in pre-medicine. After freshman year with an enthusiastic botany professor, she came to a greater appreciation for the complexity of plants as individuals and as collective communities, and decided to take up botany in hopes of understanding plant interactions. She apprenticed in plant molecular biology under Dr. Wan-Ling Chiu from the summer of 2006 through the spring of 2009 when she graduated magna cum laude from VCU in the spring of 2009. After much debate, she decided ecology could answer more questions about emergent properties, therefore sought a position that would best incorporate physiology and ecosystem ecology. Maria took a position with Dr. Taryn Bauerle at Cornell University, where they concocted experiments pertaining to root ecology of invasive species, a largely unexplored aspect of plant physiological ecology. While future plans remain uncertain, she would like to continue working to further knowledge through plant research and bring appreciation for plants to the general public who may not recognize how incredible plants really are.

This work is dedicated to all of those who have had the patience to see it to completion. It takes a long time to watch the grass grow. A special dedication to my family: Dad, "Nanny" Darlene, and brother, John. Lastly, thanks for the love and laughs, William Gibb. You are my rock, literally, my belay partner.

ACKNOWLEDGMENTS

I would like to acknowledge my committee, Dr. Taryn Bauerle, Dr. Toni DiTommaso, and Dr. Jason Fridley for their help and guidance in forming this project, helping with content, and the editorial process. To the Bauerle lab, especially Michela Centiari, Marc Goebel, Bryan Emmitt, John Zhao, and Madeline Olberg, whose help was instrumental in material processing and technical support. Thank you, as well, to William Gibb for your computational super powers for data analysis. For the help I received in maintaining my plants, I would like to acknowledge J.C. Mosher at Blue Grass Lane and Jenny Rothenberg at Kenneth Post greenhouse. Funding and financial support was obtained through Cornell University department of neurobiology and behavior, the Andrew W. Mellon foundation, Kieckhefer Adirondack fellowship program, and the New York State Flower Initiative. Lastly, I would like to acknowledge Larry of plant sciences for lifting sprits with his positive attitude on all of my earliest mornings and to the administrative staff of the horticulture department for their assistance.

TABLE OF CONTENTS

	Page
Abstract……	ii
Biographical Sketch…..……	iii
Dedication……	iv
Acknowledgements…….	v
Preface…….	vii – xi
Works Cited…….	xii – xvii
Chapter 1: "Root Dynamics of congeneric native…….	1-43
and non-native vines (*Lonicera*) and shrubs (*Rhamnus*)"	
ABSTRACT…….	1
INTRODUCTION……	2-6
METHODS AND MATERIALS…….	6-11
RESULTS…….	31-35
DISCUSSION……...	36-43
WORKS CITED……	45-52
Chapter 2: "Contrasting xylem vessel constraints on…….	44-81
hydraulic conductivity between native and non-native	
woody understory species"	
ABSTRACT……..	44

INTRODUCTION…….. 44-47

METHODS AND MATERIALS…… 47-56

RESULTS…… 56-73

DISCUSSION……. 73-77

WORKS CITED……. 78-81

Conclusion……... 82-84

WORKS CITED…….. 85-87

PREFACE

Introduced non-native species are one of the largest contributors to biodiversity loss, displacing and threatening several native plant species in natural ecosystems (Vitousek, 1988; Hiebert and Stubbendieck 1993). Non-native plants have the potential to significantly alter both community composition and important ecosystem functions such as net primary productivity, nutrient cycling and water availability (Vitousek, 1990; Naeem *et al.* 1994; Kourtev *et al*. 2002). In addition to ecosystem costs, invasive plants cause major economic losses in agriculture and forestry (Hiebert and Stubbendieck 1993; Pimentel *et al*. 2005). Woody plants, in particular, are the most abundant and widespread terrestrial invasive plants across southern New England, introduced primarily for agriculture, horticulture, and conservation (James and Drenovsky 2007; Mosher *et al*. 2009). Therefore, examining the causes of woody plant invasions remains an important and relevant topic of ecology and invasion biology.

A major goal of invasion biology is identifying attributes that contribute to competitive ability (Davis 2009). Factors reported to affect invasive success rate of plants include high fitness, growth rate, resource use efficiency, plasticity and resistance to herbivory and pathogens (Daehler 2003; Pysek and Richardson 2007; Osunkoya *et al.* 2010a; Matzek 2012). While many past studies have focused on relatively simple traits such as growth form and height, more recent studies have been examining potentially important functional traits related to invasion, such as

physiology, biomass, water use efficiency (WUE) and resource capture strategies (Osunkoya *et al.* 2010b; Leishman *et al*. 2010; Cleland 2011).

The timing of leaf emergence and senescence (phenology) is an important contributing factor to the success of non-native plant species. In deciduous forests, phenology exerts strong control over aboveground plant resource acquisition and nutrient cycling through increased aboveground foliage production (Ehrenfeld 2003; Xu *et al*. 2007; Nord and Lynch 2009). A broad range of non-native species exhibit extended leaf phenology, capturing carbon earlier in the spring and particularly late in the fall, a trait uncommon to native species (Fridley 2012). Understanding traits that enable extended leaf or root phenology is important to how plants pre-empt native plants for resource capture.

Recent meta-analyses and reviews have attempted to compile information to detect differences in traits associated with invasiveness (Lavorel and Garnier 2002; Pysek and Richardson 2007; van Kleunen *et al*. 2010; Tecco *et al*. 2010). van Kleunen *et al.* (2010) concluded that invasive plants generally exhibit higher values for performance-related traits. However, these traits are limited to aboveground performance traits such as photosynthetic rate, transpiration, leaf-area allocation and shoot allocation. Although greater than 50% of total net primary productivity can comprise root systems, the majority of invasive literature discusses only aboveground growth strategies with few reports on root relative growth rates (Caldwell 1987; James and Drenovsky 2007). Additionally, with such little knowledge of belowground systems, meta-analyses cannot extrapolate to invasive potential on a whole-plant level.

The finest, most distal root order branches in a root system are responsible for water and nutrient acquisition and inputs of carbon (C) and nutrients through decomposition into soil (Pregitzer 2002; Brassard *et al*. 2009). In contrast with higher order structural roots, low-order fine roots are characterized by higher N concentration, higher specific root length (SRL), and higher respiration rates (Pregitzer *et al.* 1998, 2002; Burton *et al*. 2012). Production and lifespan of these low-order roots are controlled by both endogenous factors (diameter, mycorrhizal associations) (Eissenstat and Yanai 1997; Guo 2008) and exogenous factors (temperature, soil moisture, nutrient availability and rooting depth), though the importance of these factors varies considerably over species and environment (King *et al.* 2002; Tierney *et al*. 2003; Guo *et al*. 2008; McCormack *et al.* 2012). Generally, fast-growing species have a higher degree of root plasticity compared to slow-growing species, suggesting that non-native plants that demonstrate a higher rate of root turnover provide greater root length to nutrient-rich microsites and allocate more biomass to roots may confer a competitive advantage in soil resource acquisition compared to slower-growing, longer-lived roots of native species (Robinson 1994; Drenovsky *et al*. 2008).

To understand the roll of fine root dynamics in relation to aboveground phenology, we non-destructively monitored fine root production and turnover of two congeneric pairs of native and non-native vines (*Lonicera*) and shrubs (*Rhamnus*) using minirhizotron observation tubes. Our objectives were to characterize root production and turnover between natives and non-natives and determine if belowground behavior mirrored aboveground leafing processes, and whether there

was a difference in root physiological activity between natives and non-natives to support extended leaf phenology.

Aboveground, general trends in species' hydraulic conductivity have been broadly categorized by means of xylem conduit diameter and rate of water flow (efficiency; Tyree 1994), and that there exists a positive relationship between vessel diameter and growth rate, such that fast-growing invasive species should exhibit wider conduits with higher water flow. However, embolism vulnerability places a constraint on maximum water transport through a reduction in hydraulic conductance as a consequence of drought and freeze-thaw cycles (Meinzer *et al*. 2001; Pockman and Sperry 1996) and even normal growth conditions (Cochard and Tyree 1990; Sperry *et al*. 1994; McCully *et al*. 1998). Yet few, if any studies, on the relationship between vessel conduit anatomy and water transport in native versus non-native species have been performed to date. Likewise, comparative studies that investigate vessel characteristics over a wide survey of plant species are limited (but see McCulloh *et al*. 2010; Jansen *et al*. 2004). In a second study, we tested the hypothesis that non-native understory species have greater capacity for water transport than native species through examining hydraulic properties of 83 native and non-native woody species common to forests of Eastern North America, including several congeneric groups, which represent a range of anatomical wood types. We explored relationships between stem xylem vessel anatomical arrangement, xylem vessel types, stem specific hydraulic conductivity, and cavitation vulnerability among native and non-native woody understory species.

Works Cited:

Brassard B.W., Chen H.Y.H., and Bergeron Y. (2009) Influence of environmental variability on root dynamics in northern forests. *Critical Reviews in Plant Science*. 28: 179-197.

Burton A.J., Jarvey J.C., Jarvi M.P., *et al.* (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biology*. 18: 258-266.

Caldwell M. (1987) Competition between root systems in natural communities. Society for Experimental Biology Seminar Series: Root Development and Function. New York, New York, USA. Cambridge University Press.

Cleland E. (2011) Trait divergence and the ecosystem impacts of invading species. *New Phytologist.* 189: 649-652.

Cochard H. and Tyree M.T. (1990) Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiology*. 6: 393-407.

Daehler C. (2003) Performance comparisons of co-occuring native and alien invasive plants: implications for conservation and restoration. *Annual Review Of Ecology Evolution and Systematics*. 34: 183-211.

Davis M. (2009) *Invasion Biology*. New York, New York, USA. Oxford University Press Inc.

Eissenstat D.M. and Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research.* 27: 1-60.

Ehrenfeld J.G. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*. 6: 503-523.

Fridley J. (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. *Nature*. 485: 359-362.

Guo D., Mitchell R.J., Withington J.M., Fan P-P., and Hendricks J.J. (2008) Endogenous and exogenous controls of roots life span, mortality and nitrogen flux in a longleaf pine forest: foot branch order predominates. *Journal of Ecology*. 96: 737-745.

Hiebert R.D. and Stubbendieck J. (1993) Handbook for Ranking Exotic Plants for Management and control. U.S. Department of Interior, National Park Service, Denver, CO.

James J.J. and Drenovsky R.E. (2007) A basis for relative growth rate differences between native and invasive forb seedlings. *Rangeland Ecol. Manage.* 60: 395-400.

Jansen S., Baas P., Gasson P., *et al*. (2004) Variation in xylem structure from tropics to tundra: Evidence from vestured pits. *Proceedings of the National Academy of Sciences*. 101: 8330-8837.

King J.S., Albaugh T.J., Allen H.L., *et al*. (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist*. 154: 389-898.

Kourtev P., Ehrenfeld J., and Haggblom M. (2002) Exotic plant species alter the microbial community structure and function in the soil. *Ecology*. 83: 3152-3166.

Lavorel S. and Garnier E. (2002) Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*. 16: 545-565.

Leishman M., Michelle R., Thomson V.P., and Cooke J. (2010) Native and exotic invasive plants have fundamentally similar carbon capture strategies. *Journal of Ecology*. 98: 28-42.

Matzek V. (2012) Trait values, not trait plasticity, best explain non-native species' performance in a changing environment. *PLoS ONE*. 7: e48821.

McCormack M.L., Adams T.S., Smithwick E.A.H., and Eissenstat D.M. (2012) Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist*. 195: 823-831.

McCulloh K., Sperry J.S., Lachenbruch B., *et al*. (2010) Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. *New Phytologist*. 186: 439-450.

McCully M.E., Huang C.X. and Ling L.E.C. (1998) Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist* 138: 327-342.

Meinzer F.C., Clearwater M.J. and Goldstein G. (2001) Water transport in trees: current perspectives, new insights and some controversies. *Environmental and Experimental Botany*. 45: 239-262.

Mosher E.S., Silander J.R., and Latimer A.M. (2009) The role of land-use history in

major invasions by woody plant species in the northeastern North American landscape. *Biological Invasions*. 11: 2317-2328.

Naeem S., Thompson L.J., Lawler S.P., *et al*. (1994) Declining biodiversity can alter the performance of ecosystems. *Nature*. 368: 734-737.

Osunkoya O.O., Bayliss D., Panetta F.D., *et al*. (2010a) Variation in ecophysiology and carbon economy of invasive and native woody vines of riparian zones in south-eastern Queensland. *Austral Ecology*. 35: 636-649.

Osunkoya O.O., Bayliss D., Panetta F.D., *et al*. (2010b) Leaf trait co-ordination in relation to construction cost, carbon gain and resource-use efficiency in exotic invasive and native woody vine species. *Annals of Botany*. 106: 371-380.

Pimentel D., Zuniga R. and Morrison D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*. 52: 273-288.

Pregitzer K.S., Laskowski M.J., Burton A.J., *et al*. (1998) Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology*. 18:665-670.

Pregitzer K.S. (2002) The fine roots of trees – a new perspective. *New Phytologist*. 156: 267-270.

Pockman W.T. and Sperry J.S. (1996) Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia*. 109: 19-27.

Pysek P. and Richardson D. (2007) Traits assocatied with invasiveness in alien plants: where do we stand? *Biological Invasions*. 193: 97-125.

Sperry J.S., Nichols K.L., Sullivan J.E.M. and Eastlack S.E. (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of Northern Utah and interior Alaska. *Ecology*. 75: 1736-1752.

Tecco P.A., Diaz S., Cabido M., *et al*. (2010) Functional traits of alien plants across contrasting climatic and land-use regimes: do aliens join the locals or try harder than them? *Journal of Ecology*. 98: 17-27.

Tierney G.L., Fahey T.J., Groffman P.M., *et al.* (2003) Environmental control of fine root dynamics in a northern hardwood forest. *Global Change Biology*. 9: 670-679.

Tyree M.T., Davis, S.D., and Cochard H. (1994). Biophysical perspectives of xylem evolution – is there a trade-off of hydraulic efficiency for vulnerability to dysfunction. *Iawa Journal*. 15:335-360.

van Kleunen M., Weber E., Fischer M., *et al*. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*. 13: 235-245.

Vitousek P.M. (1988) Diversity and biological invasions of Oceanic Islands. In: Wilson E.O., Peter F.M. (Eds.), Biodiversity. National Academy of Sciences, Washington, DC, pp.181-189.

Vitousek P.M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos*. 57: 7-13.

Wooley S.C., Smith B., King C., *et al*. (2011) The lesser of two weevils:

physiological responses of spotted knapweed (*Centaurea stoebe*) to above- and belowground herbivory by *Larinus minutus* and *Cyphocleonus achates*. *Biocontrol Science and Technology*. 21: 153-170.

CHAPTER 1

ROOT DYNAMICS OF CONGENERIC NATIVE AND NON-NATIVE VINES (*LONICERA*) AND SHRUBS (*RHAMNUS*)

Abstract

Belowground processes are rarely considered in studies of the biology of non-native invasive species, which are widely recognized to alter community and ecosystem processes through differences in leaf phenology and aboveground leaf production. We examined relationships between belowground fine root production, lifespan, leaf phenology, and seasonal nitrogen uptake in two pairs of congeneric species, including. *Lonicera japonica* (non-native), *L. semeprvirens* (native), *Rhamnus frangula (non-native)*, and *R. alnifolia (native),* using minirhizotron technology and mylar windows. Across seasons, there was a significant difference in fine root production between *Lonicera* species but not those of *Rhamnus*. *L. japonica* had higher fine root production during spring and fall while *L. sempervirens* produced the most roots during summer. The timing of root and leaf production coincided within each species, but there was no correlation between leaf and fine root production. Root life span differed within *Lonicera* and *Rhamnus* species, with root order and the number of neighbors at the time of root death as the strongest predictors of root lifespan. Root life span was generally shortest during the spring and longest during the fall. Roots of *L. japonica* had consistently shorter lifespans compared to *L. sempervirens*, but no pattern was found for *Rhamnus* species. Seasonal ^{15}N uptake was higher in spring than in the fall, as expected. Although there was no difference in uptake between *Rhamnus* species, our results suggest higher and faster spring ^{15}N uptake in non-native *L. japonica* compared to native *L. sempervirens*. Overall, our results indicate the potential for the fast-growing non-native *Lonicera* japonica to outcompete native

species through differential timing of root production, root turnover, and nitrogen uptake, but limited evidence that this is a general strategy of invader dominance.

Introduction

Non-native plant species have the potential to significantly alter both community composition and ecosystem processes such as net primary productivity, nutrient cycling, and water availability (Naeem *et al*. 1994; Vitousek 2000; Kourtev *et al*. 2002), as one of the largest contributors to global biodiversity loss (Vitousek 1988). Recently, many efforts have focused on identifying plant traits to understand the underlying process of plant invasions, including differentials in growth rate and size, reproduction rate, and physiological traits inherent to successful invaders (van Kleunen *et al*. 2010; Drenovsky *et al*. 2012).

An important factor contributing to the success of non-native plant species is how they capture resources, including the timing of leaf emergence and senescence. In deciduous forests, growth phenology exerts strong control over aboveground plant resource acquisition and nutrient cycling through increased aboveground foliage production (Ehrenfeld, 2003; Xu *et al*. 2007). Fridley (2012) demonstrated that a broad range of non-native species exhibit extended leaf phenology, capturing carbon earlier in the spring and particularly later in the fall, an uncommon attribute in native species. Along with contrasting phenology, many fast-growing, non-native species exhibit leaf-level traits such as high specific leaf area (SLA) and higher foliar nitrogen and phosphorous content (van Kleunen *et al*. 2010; Heberling and Fridley 2013). These traits typically correspond to faster plant growth strategies and are negatively correlated with leaf lifespan (LL) (Leishman *et al*. 2007; Wright *et al*. 2004).

The capacity for high plant relative growth rate (RGR) is often credited for the success of non-native woody plants during the establishment phase following colonization (Pysek and

Richardson 2007; Tecco *et al*. 2010; Matzek 2012), particularly under high-resource, low-stress conditions (Daehler 2003). Invasion in low-resource environments also occurs, though with less frequency than high-resource environments (Davis *et al*. 2000; Funk and Vitousek 2007). To effectively compete in areas where native plants have evolved to efficiently utilize limited resources, fast-growing non-native plants must either persist in the environment through resource use efficiency adjustment (Funk and Vitousek 2007; Heberling and Fridley 2013), increasing resource availability through N-fixation (Kurten *et al*. 2008) or positive-feedback through litter decomposition (Eherenfeld *et al*. 2001; Kourtev 2003; Allison and Vitousek; 2008; Trammell *et al.* 2012). Additionally, many aboveground traits generally common to fast-growing non-natives exhibit morphological and/or physiological plastic responses to fluxes in light availability (Funk 2008; Matrina and von Ende 2012). Despite the importance in resource acquisition and nutrient cycling, analogous morphological and physiological root traits of native and non-native plants are much less understood (Comas and Eissenstat 2004; Kattge *et al*. 2011).

The finest, most distal root order branches in a root system are responsible for water and nutrient acquisition and inputs of carbon (C) and nutrients through decomposition into soil (Pregitzer 2002; Brassard *et al*. 2009). In contrast with higher order structural roots, low-order fine roots are characterized by higher N concentration, higher specific root length (SRL), and higher respiration rates (Pregitzer *et al.* 1998, 2002; Burton *et al*. 2012). Production and lifespan of these low-order roots are controlled by both endogenous factors (diameter, mycorrhizal associations) (Eissenstat and Yanai 1997; Guo 2008b) and envrionmental factors (temperature, soil moisture, nutrient availability and rooting depth), though the importance of

these factors varies considerably over species and environment (King *et al.* 2002; Tierney *et al*. 2003; Guo *et al*. 2008b; McCormack *et al.* 2012).

Although controls on leaf phenology are well understood (Cleland *et al.* 2007), we have much less knowledge of controls on root phenology and the relationship between root and leaf phenology. Previous studies on temperate tree species found root production and mortality are highly synchronized with foliar production, where root systems are expanded prior to leaf growth in order to support necessary water and nutrient uptake (Lyr and Hoffmann 1967; Hendrick and Pregitzer 1993; King *et al*. 2002). However, periodicity of root production is strongly contingent upon environmental conditions. In Northern hardwood forests under unlimited water conditions, fine root production has been shown to occur slowly in spring and peak mid-summer before declining in fall (Burke and Raynal 1994; Burton *et al*. 2000; Tierney *et al.* 2003), whereas peak fine root production generally occurs in spring and/or fall under late-summer water limitation (Hendrick and Pregitzer 1997; Joslin *et al*. 2001). Both root production and mortality are usually low over winter due to frozen soils that prohibit water uptake and microbial activity (Tierney *et al.* 2003). Understanding environmental controls on root phenology is necessary to predict how introduced non-native species may respond to new environmental conditions.

Several attempts have been made to link above and belowground growth phenology and lifespan. Joslin *et al*. (2001) found a relationship between the production of elongating roots (root elongation intensity – REI) with leaf phenology, where the highest REI levels coincided with active foliage expansion and growth in mature oak trees between April and early August. Likewise, Steinaker and Wilson (2008) found a negative correlation in the timing of peak leaf and root production in aspen forests, with peak root production occurring 45 days after leaf

production. Withington *et al*. (2006) compared lifespans of roots and leaves among species of varying growth rates, and found no linkage in longevity between leaves and roots. However, a significant correlation between root N concentration, root N:C, and longevity was found, supporting evidence for a linkage between root traits and lifespan. McCormack *et al*. (2012) further supported this linkage with correlations of various root traits to median lifespan across 12 species of temperate trees, with diameter, calcium content and tree wood density positively correlating with lifespan, SRL, and N:C ratio, and plant growth negatively correlating with lifespan . For non-native species, precise timing of fine root production during early spring has important implications for potential co-opting of ephemeral resources by early leafing invaders, while extended root production into autumn months could explain continued support for extended leaf production. Additionally, knowledge of root lifespan can be coupled to aboveground traits of non-native plants for greater precision in predicting non-native potential for invasive growth and their ability to alter ecosystem processes.

In this study, we explored root production, life span, and seasonal nitrogen uptake of the Eastern U.S. native forest understory species *Lonicera sempervirens* L. (vine; Caprifoliaceae) and *Rhamnus alnifolia* L'Hér. (shrub; Rhamnaceae), and their non-native invasive congeners *Lonicera japonica* Thunb. and *Rhamnus frangula* Mill.. These four species demonstrated strong contrasts in leaf phenology and lifespan among over 70 species from seven different genera in a common garden study (Fridley 2012). We examined the relationship of aboveground to belowground growth phenology between native and non-native pairs, with the expectation that non-natives exhibit both earlier and later root production corresponding with aboveground leaf phenology. In addition, we hypothesized that non-native species would have shorter-lived roots compared to native species, the latter showing longer root lifespan for roots

born during the spring season. We also expected root order to be a more significant predictor of root life span, rather than root diameter, such that higher order root branches have longer lifespans than lower order branches, as suggested by McCormack (2012) and Guo *et al*. (2008b). Lastly, we compared root nitrogen uptake, using isotopic ^{15}N, over fall and spring seasons between congeneric pairs to determine whether there was a difference in root physiological activity between natives and non-natives to support extended leaf phenology, and whether one advantage of non-native plants over natives is faster N uptake.

Material & methods

Leaf phenology

Three replicate individuals of *L. japonica*, *L. sempervirens*, *R. frangula*, and *R. alnifolia* were established between 2006 and 2007 in a common garden plot in Syracuse, New York (USA). Individuals were maintained under 80% shade from mid-May through mid-October, simulating canopy closure and understory light conditions. Leaf phenology and demography was collected biweekly over the 2008 through 2010 growing seasons, following five branches per individual monitored for the timing of spring foliar bud and leaf development and biweekly leaf production counts (Fridley 2012). 2008 data was chosen from data between 2008 through 2010. Data collected from 2008 and 2009 were very similar, while phenology data for 2010 showed much earlier full leaf emergence due to a warmer than average spring (Fridley 2012). Demography data, however, did not differ between the three measured years. Leaf lifespan was measured as an average across the growing season, calculated as the area beneath the total live leaves curve divided by the total number of emerged leaves (Kikuzawa 1995, Fig. 1).

Root production and lifespan

Separate individuals were established to examine root behavior and nutrient uptake. *L. japonica, L. sempervirens* and *R. alnifolia* were propagated using 0.8% IBA in talc (Hormodin® 3, OHP Inc.) from hardwood cuttings of established individuals in November 2009. Cuttings of *R. alnifolia* were obtained from Reeseville Ridge Nursery, Reeseville, WI due to lack of sufficient propagation material. In May 2010, seven replicates of each species were transferred to 18-gallon pots filled with a 50-50 mix of perlite and sterilized Hudson silty clay loam soil (pH 7.5) excavated from the Blue Grass Lane field site in Ithaca, NY. All pots contained replaceable mylar observation 'windows' (250 x 336mm) to provide access to undisturbed attached roots. Aluminum insulation was used to cover the windows to prevent light penetration into the root box.

Plants were arranged in a completely randomized design. During periods of full forest canopy enclosure, from mid-May through mid-October, plants were maintained under knitted black 80% shade cloth (Dewitt Company Inc., Sikeston, MO, USA) to mimic natural growing conditions. Plants were overwintered in a neighboring polyhouse for the first winter, and were maintained in surrounding mulch for the following two winters. All plants were watered during summer months.

Root Observations and Measurements

Acrylic minirhizotron root observation tubes (50.5 cm long, 6 cm outer diameter) were installed in May 2010 at a 30° angle from the vertical. Two minirhizotron tubes were installed per box, equidistant from each side of the plant. Each species had a total of 14 observation tubes, for 56 tubes total. Ten centimeters of each tube was left above the soil surface, wrapped in black electrical tape and capped with rubber stoppers to prevent light penetration. White

metal cans were placed over the top of the tube to minimize radiant heating, and PVC plastic plugs were used to prevent water from entering the bottom of the tubes.

Root image observations were collected along each tube in one-week intervals during the growing season (May through September) and once monthly during the dormant periods (October through April) using Bartz I-CAP image capture system (Bartz Technology, Santa Barbara, CA, USA). Images captured measured 14 mm in height and 18 mm in width. All images were analyzed for root population counts, seasonal root production, and survivorship using WinRhizo Tron MF (Regents Inc., Quebec, Canada). Root births and root deaths were estimated by calculating the date midway between the birth observation date and death and the previous observation date. A root was considered censored unless it disappeared from the viewing area. Black and white image color did not allow for distinction between a live versus pigmented root. Roots appearing in continuous frames within the same tube were only counted once. A topological approach was applied to classifying root order, where roots terminating in a meristem, identified as a root tip, with no dependent laterals were considered first order, roots with only one lateral were considered second order, and so forth (Fitter 1987; Pregitzer *et al*. 2002). Only first and second order roots were counted due to the low sample size of third order roots (less than 10) visible for each species.

Differences in root lifespan were analyzed using Cox proportional hazards regression (PROC PHREG, Enterprise Guide 5.1, SAS Institute, Inc., Cary, NC, US). Cox proportional hazards allow the evaluation of each covariate while controlling for the effects of other covariates (Cox 1972). In this analysis, individual roots are evaluated for their 'hazard', the risk of mortality of a root over time t, where t is the product of a baseline hazard function of k covariates (Allison 1995). The partial likelihood method from PROC PHREG estimates a β

coefficient for each covariate (Cox 1972), and the model calculates a chi-square statistic to test the null hypothesis that each β is equal to zero. Parameter estimates can be positive or negative, *e.g.* a negative β indicating a decreased hazard of mortality (longer root lifespan) as the covariate increases (Wells and Eissenstat 2001; Guo *et al*. 2008b). Covariates in the root survivorship model included root diameter and root neighbors (the number of roots present in the image window), and root order. Roots were additionally analyzed for differences in survivorship by season of birth. Roots born during winter months were excluded from the analysis and the remaining roots were pooled due to insufficient number of roots. The hazard ratio (e^{β}) was interpreted based on classification of covariates as either categorical or continuous (Allison 1995; Guo *et al*. 2008b). For example, the hazard ratio of a categorical covariate such as "root order" would be interpreted as the ratio of the hazard of a first order root (1) to that of a second order root (2), controlling for all other covariates. For a quantitative covariate such as root diameter, the estimated percent change in the hazard associated with one unit of change in the covariate would be one subtracted from the hazard ratio and multiplied by 100 (Allison 1995). Seasonal root production was calculated as the percent of fine roots produced by each species seasonally as a proportion of the total. Standing crop biomass was calculated as the difference between cumulative production and cumulative mortality of fine roots.

^{15}N Uptake Experiment

Seasonal individual fine root nitrogen uptake was measured using the ^{15}N tracer method in October 2010 and May 2011. Root growth was tracked daily for four weeks prior to ^{15}N sampling using different colored paint markers to ensure precise root age. New white root tips

less than three days old without lateral branching were used to track declining nitrate uptake with increasing root age (as in Volder *et al.* 2005).

Prior to measurements, small cuts were applied to mylar windows to access root tips and keep them intact as well as to clean the tips from soil particles. The lid of the Eppendorf tubes was punctured for root insertion and to minimize solution evaporation from the tube. Root tips were placed overnight into a buffer solution of 0.6 mL with a pH 5.7, containing 0.5 mL of 1mM, unlabeled KNO3 buffered with 10 mM MES, 1 mM $CaSO_4$, and 4 uM K_2HPO_4 (Volder 2005). Three to four new fine roots for three different time periods (zero, three, six hours) were used from each plant to test for nitrate uptake, using labeled $K^{15}NO_3$ (98 At %, Sigma Aldrich, St. Louis, MO, USA), as well as an unlabeled KNO_3 as control. The following day, roots were placed into a new vial containing 1 mM labeled $K^{15}NO_3$ for zero, three or six hour time periods. All controls were also transferred to fresh tubes containing the 1 mM unlabeled buffer solution for six hours. The mylar windows on the front of the root box remained covered, with the majority of root still in the soil, with the exception of the portion of roots exposed to the tube. At the end of the zero, three, and six hour time period, roots were excised, rinsed in deionized water and placed into aluminum tins of a 96-well plate to dry at 60 °C for 12 hours. Dried tissue samples in aluminum tins were weighed and analyzed for $\delta^{15}N$ enrichment using an elemental analyzer (EA-IRMS) at the Cornell University Stable Isotope Laboratory (COIL, Ithaca, NY). Gross N-uptake was calculated from the % ^{15}N in root tissue, expressed as in μg of ^{15}N per μg of root tissue. Using pooled root data for each species, a mixed-effects model was constructed to test the effects of hours of ^{15}N exposure, season, native status and interactions on fine root ^{15}N concentrations in JMP (SAS Institute Inc, Cary, NC, v.

10.0). Additionally, individual models were constructed to test for effects of time of native status within individual seasons.

Results

Leaf phenology and demography

Full leaf expansion of non-native *L. japonica* occurred eight days prior to *L. sempervirens*, while 90% of leaf fall occurred for *L. japonica* five days prior to *L. sempervirens,* (Fig. 1). The two *Lonicera* species had large differences in leaf life span, with an average of 38 days for *L. japonica* compared to 120 days for *L. sempervirens* (data not shown). The mean total leaves produced per new annual shoot by *L. japonica* (257 leaves) significantly exceeded those of *L. sempervirens* (31 leaves, $P = 0.005$),

Rhamnus species had a larger contrast in fall leaf retention compared to *Lonicera* species. While the date of full leaf expansion within *Rhamnus* was the same for both species, April 23rd, the date of 90% leaf drop was approximately one month later for non-native *R. frangula*, occurring on November 11th compared to October 9th for native *R. alnifolia*. Leaf life span was similar between *Rhamnus* at 114 days in *R. frangula* and 110 days in *R. alnifolia* (data not shown). No difference in total leaf production per shoot was seen between non-native *R. frangula* and native *R. alnifolia* (14 leaves and 9 leaves, respectively, $P = 0.164$).

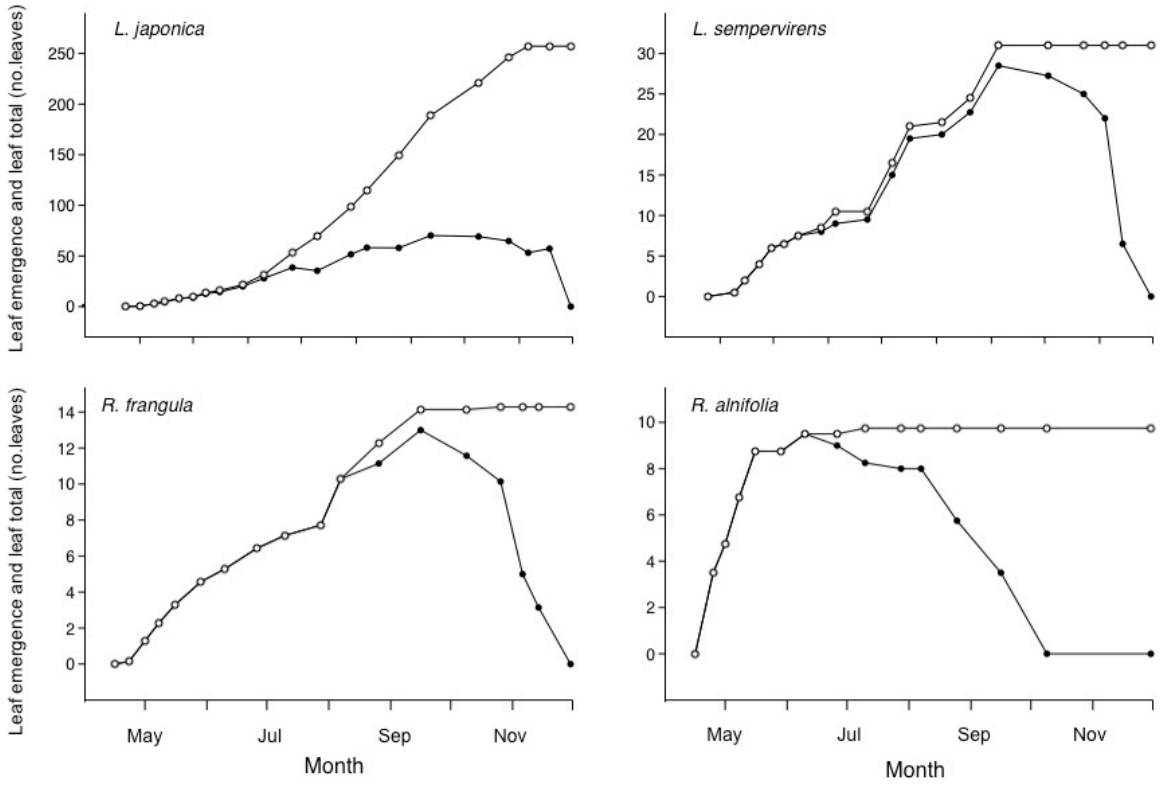

Figure 1 Leaf demography of congeneric native and non-native species growing in a common garden over the 2008 growing season: a) *Lonicera japonica,* b) *Lonicera sempervirens,* c) *Rhamnus frangula*, and d) *Rhmamnus alnifolia*. Leaf count measurements stopped being taken at 25% leaf fall. Black circles (●) indicate total live leaves per survey and open circles (○) indicate cumulative leaf emergence. Note the difference in y-axis scale. (n = 15 branches)

Root Diameter

For all four species, observed first and second order root diameters were 1.01 mm to 8.4 mm, with most root diameters falling between 1-3 mm (Fig. 2). Median diameter of both first and second order roots was smaller for each root order in *L. japonica* compared to *L. sempervirens* ($P < 0.01$). In *Rhamnus*, however, *R. frangula* had larger median root diameters for both first and second order roots compared with *R. alnifolia* ($P < 0.0001$).

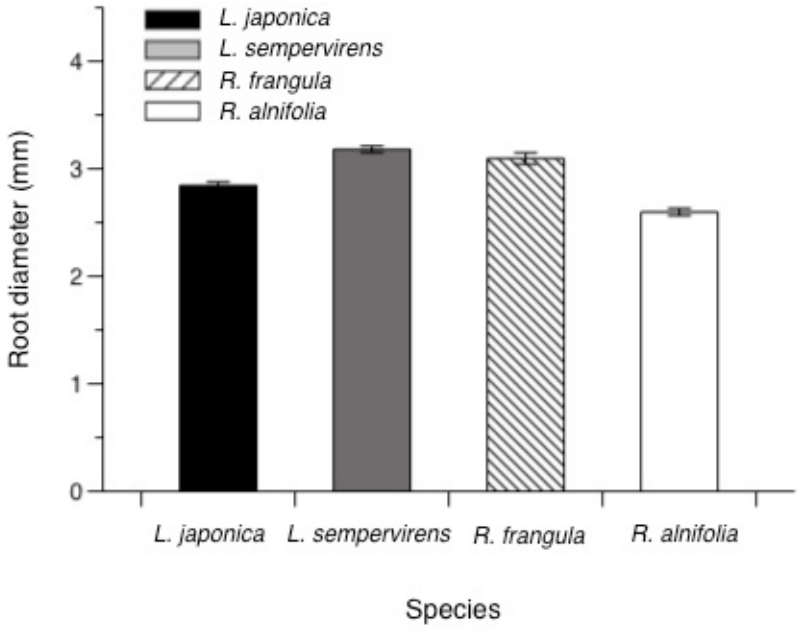

Figure 2 Average root diameter (mm) first and second order roots of *L. japonica*, *L. sempervirens*, *R. frangula* and *R. alnifolia*. Bars represent ±1 SE.

Seasonal root production

Native species had a higher total root production of fine roots compared to non-native congeners between July 2010 and February 2012, with *L. sempervirens* having the highest total production over the 704 days (794 roots, 43.33%, Table 1), although differences between *Rhamnus* were not significant. The highest seasonal root production across all four species occurred during summer (June to August, 53.3%, Table 1) and fall (September to November, 26.04%, Table 1, Fig. 3). Few roots were produced during winter (December to February, 4.64%).

Table 1 Results of Chi-square test for differences in total fine root production between
congeneric pairs of *Lonicera* and *Rhamnus*

Species	No. roots / season (*Sp, Sm, Fa, Wn)	Proportion of total	Total no. roots	χ^2	*P*
L. japonica	109	26.6	410	48.337	<0.0001*
	134	32.7			
	142	34.6			
	25	6.1			
L. sempervirens	132	16.6	794		
	425	53.6			
	199	25.1			
	38	4.8			
R. frangula	22	7.2	307	2.712	0.4381
	207	67.4			
	70	22.8			
	8	2.6			
R. alnifolia	30	9.4	321		
	211	65.7			
	66	20.56			
	14	4.4			

* Sp = spring, Sm = summer, Fa = Fall, Wn = Winter

Significant differences in fine root production were detected between *Lonicera* species (Table 1, $P < 0.0001$). *L. japonica* produced a higher proportion of fine roots in the spring and fall relative to *L. sempervirens*, with 26.6% and 34.6% compared to 16.6% and 25.1% of total production, respectively (Fig 3). Differences in fine root production between *Rhamnus* species were less pronounced ($P = 0.438$). *R. alnifolia* produced more roots in spring and winter, and *R. frangula* produced more roots during summer and fall (67.4%, 22.8% and 65.8%, 20.56% respectively), although this difference was not significant ($P > 0.05$).

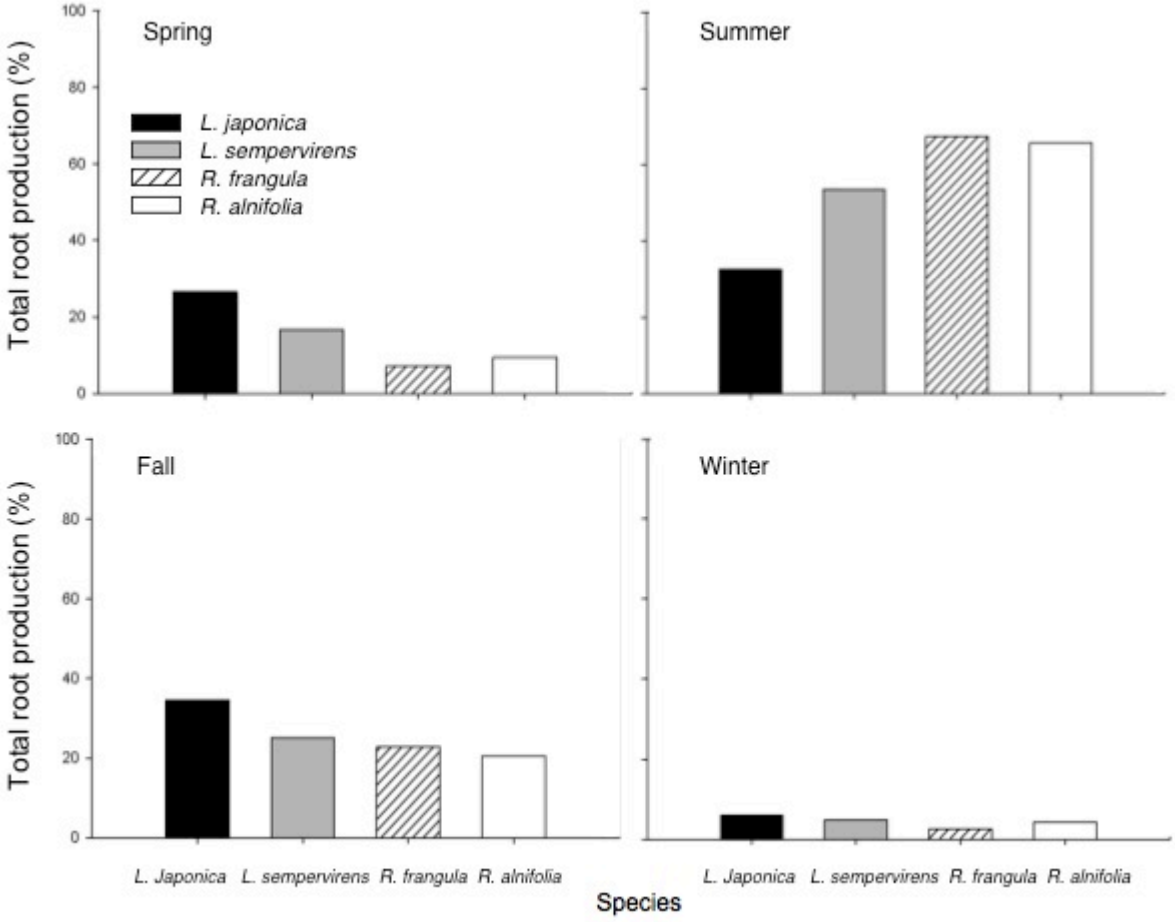

Figure 3 Root production of congeneric *L. japonica*, *L. sempervirens*, *R. frangula*, and *R. alnifolia* over each season as a percentage of the total roots produced by each species from 2010 to 2012. Spring = March to May, Summer = June to August, Fall = September to November, Winter = December to February.

Root Standing Crop

L. sempervirens had a higher root standing crop (root biomass) compared to *L. japonica* (Fig. 4), due to the high number of roots viewed on the first day of observation (Fig. 4a). *L. sempervirens* showed higher root mortality during fall and winter, whereas *L. japonica* had higher root mortality during late spring and summer (Fig. 5).

Root standing crop did not differ between *Rhamnus* species (Fig. 4b). However, *R. alnifolia* had higher fine root mortality over the summer months, while the *R. frangula* had consistent mortality across summer, fall, and winter, and low mortality during spring (Fig. 5).

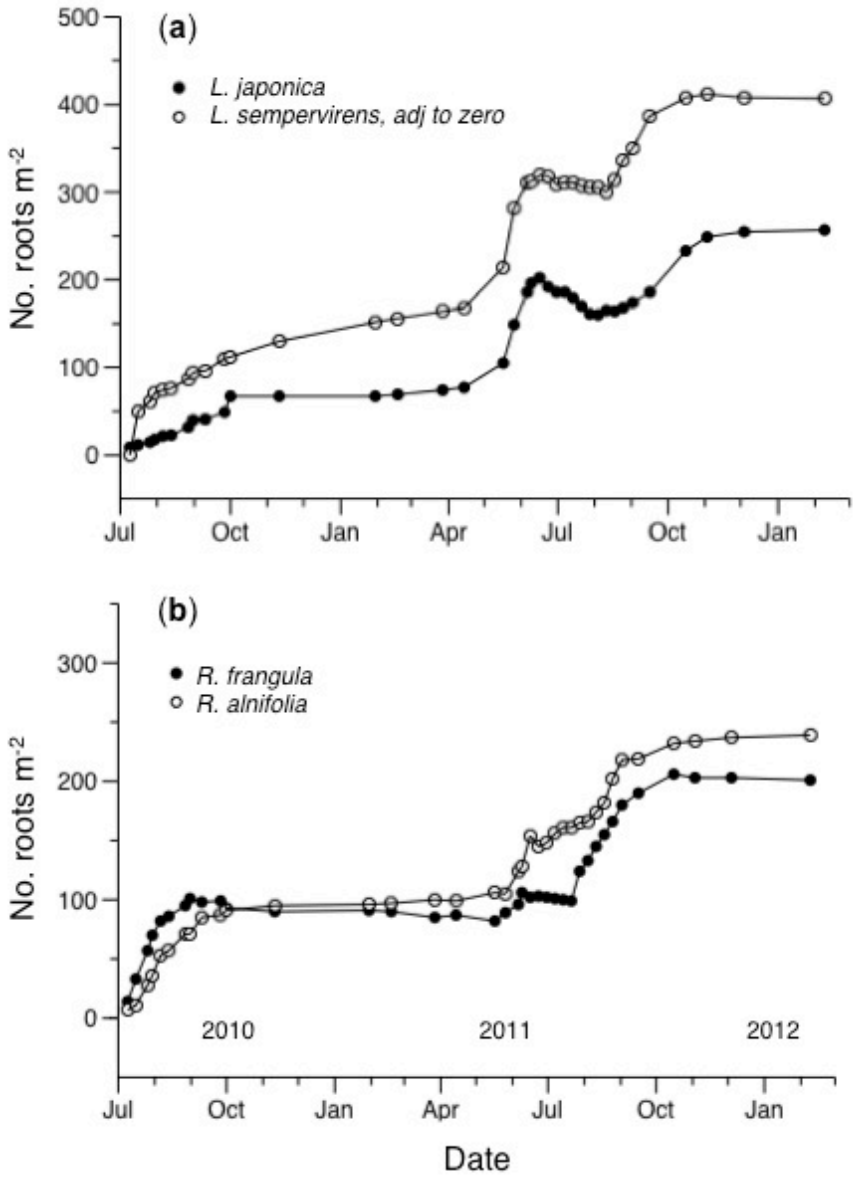

Figure 4 Cumulative root standing crop of congeneric *L. japonica*, *L. sempervirens*, *R. frangula*, and *R. alnifolia*. Data represent number of first and second order fine roots, expressed on an m^2 area, from July 2010 till March 2012. Values are calculated as cumulative root mortality subtracted from cumulative roots produced. Closed circles (●) represent non-native species, and open circles (○) represent natives. (a) Shows *Lonicera* species (b) shows *Rhamnus* species. Root production in *L. sempervirens* has been adjusted to zero. Note the difference in y-axis scales.

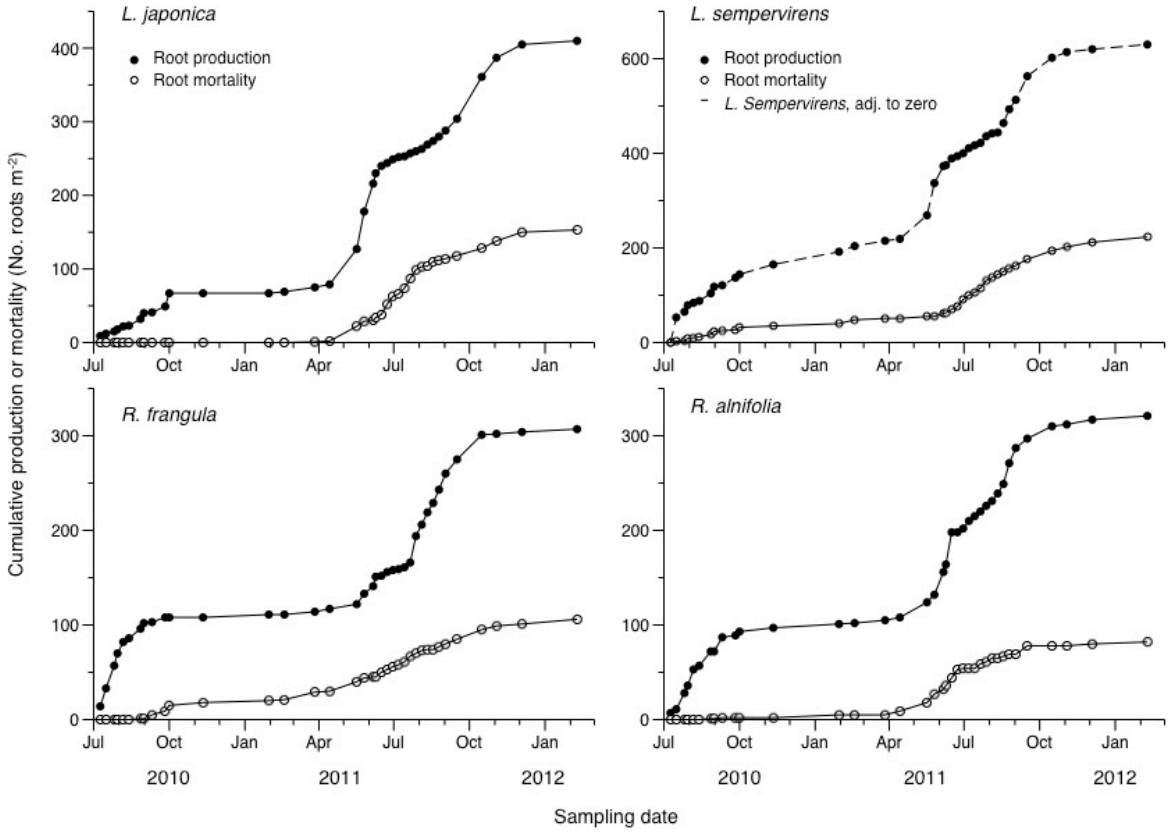

Figure 5 Cumulative root production and mortality for *Lonicera sp.* and *Rhamnus sp.* Closed circles (●) represent cumulative root production as the number of roots per mm^2, and open circles (○) represent cumulative root mortality as the number of roots per mm^2. The third dashed line in *L. sempervirens* represents the zero-adjusted value for its root production. Note the difference in y-axis scales.

Root lifespan

The following covariates had significant relationships with root lifespan in *Lonicera* species: species, root order, and the number of neighboring roots at the time of death (Table 2). *L. sempervirens* had a 180.8% ([2.808 – 1] x 100%) longer fine root lifespan in comparison to *L. japonica* ($P < 0.0001$), although both species had > 50% fine root survivorship at the end of the study period (Table 2). Lifespan of first order roots decreased by 215% compared with second order roots ($P < 0.0001$), and lifespan of roots decreased by 171.8% ([$e^{0.2496}$ – 1] x 100%) with an increase in neighboring roots at the time of root death. Root diameter was not a significant influence on root lifespan for *Lonicera* species ($P = 0.059$).

Similarly for *Rhmanus,* the following covariates, species, root order, and the number of neighbors present at the time of death, were significant predictors of root life span (Table 2). Fine root life span of *R. frangula* was 35.3% shorter compared with native *R. alnifolia (P* = 0.0036). Root lifespan increased by 74% between first and second order roots ($P = 0.0105$), and decreased by 172% for each additional neighboring root present at the time of root death ($P < 0.0001$). Root diameter did not have a significant influence on root life span for either *Rhamnus* species ($P = 0.839$).

Fine roots born in spring, summer, and fall were significantly longer-lived in *L. sempervirens* compared to fine roots of *L. japonica* ($P < 0.0001$, Fig. 6a-c). Fine roots reached median survivorship in *L. japonica* at 495 days for roots born in spring and 476 days for roots born in summer; fine roots reached survival at the 75th percentile at 213 days for roots born during the fall. No significant difference in median lifespan was found for roots born during spring and summer ($P = 0.468$). Fine roots of *L. sempervirens* reached survival at 75th percentile

at 551 days for roots born in spring and 516 days for roots born in fall. Fine roots born during summer remained visible even longer, reaching 88% survival at 551 days.

Differences in fine root lifespan between *Rhamnus* species were detected in spring and summer (Fig. 6d-f). Fine roots of native *R. alnifolia* were significantly longer-lived when born in the spring ($P = 0.007$) and summer season ($P = 0.002$) compared to non-native *R. frangula*. While fine roots of *R. alnifolia* were shorter-lived compared to *R. frangula* during the fall season, the difference in lifespan was not significant ($P = 0.299$). Fine roots of non-native *R. frangula* reached median survival time of 408 days for roots born during spring, the 75th percentile at 580 days for roots born during the summer, and reached 97% survivorship at 559 days for roots born during the fall, indicating an increase in lifespan across seasons from spring to fall. Fine roots of native *R. alnifolia* reached a final survivorship of 80% at 675 days for roots born during spring, 75th percentile survival at 675 days for roots born during the summer, and the 75th percentile survival at 208 days for roots born during the fall. Roots of *R. alnifolia* were longest-lived during the spring season, and shortest-lived during the fall.

Table 2 Number of roots with an event or censoring by season for each species (*L. japonica, L. sempervirens, R. frangula, R. alnifolia*)

# of roots	with event	censored	total	with event	censored	total
Species		*L. japonica*			*L. sempervirens*	
Season						
Spring	59	47	106	38	93	131
Summer	66	65	131	137	281	418
Fall	27	115	142	38	159	197
Species		*R. frangula*			*R. alnifolia*	
Season						
Spring	11	8	19	7	20	27
Summer	82	120	202	51	155	206
Fall	9	60	69	17	49	66

Table 3 Proportional hazards regression analysis results for root life span of congeneric natives (*L. sempervirens* and *R. alnifolia*) and non-natives (*L. japonica* and *R. frangula*)

Variable	df	Parameter estimate	Std. error	χ^2 value	P-value	Hazard ratio
Lonicera						
Neighbor	1	0.250	0.012	408.885	**<0.0001**	1.283
Species						
L. japonica	1	1.033	0.112	84.87	**<0.0001**	2.808
Root order		-	-	26.16	**<0.0001**	-
1st order	1	1.147	0.224	26.160	**<0.0001**	3.15
Diameter	1	-	-	3.567	0.059	-
Rhamnus						
Neighbor	1	0.289	0.020	217.125	**<0.0001**	1.335
Species		-	-	8.492	**0.0036**	-
R. alnifolia	1	-0.436	0.150	8.492	**0.0036**	0.647
Root order		-	-	6.549	**0.0105**	-
1st order	1	0.554	0.216	6.549	**0.0105**	1.74
Diameter	1	-	-	0.0412	0.8391	-

Significant values are denoted in bold
Non-significant parameters were removed from the regressions model

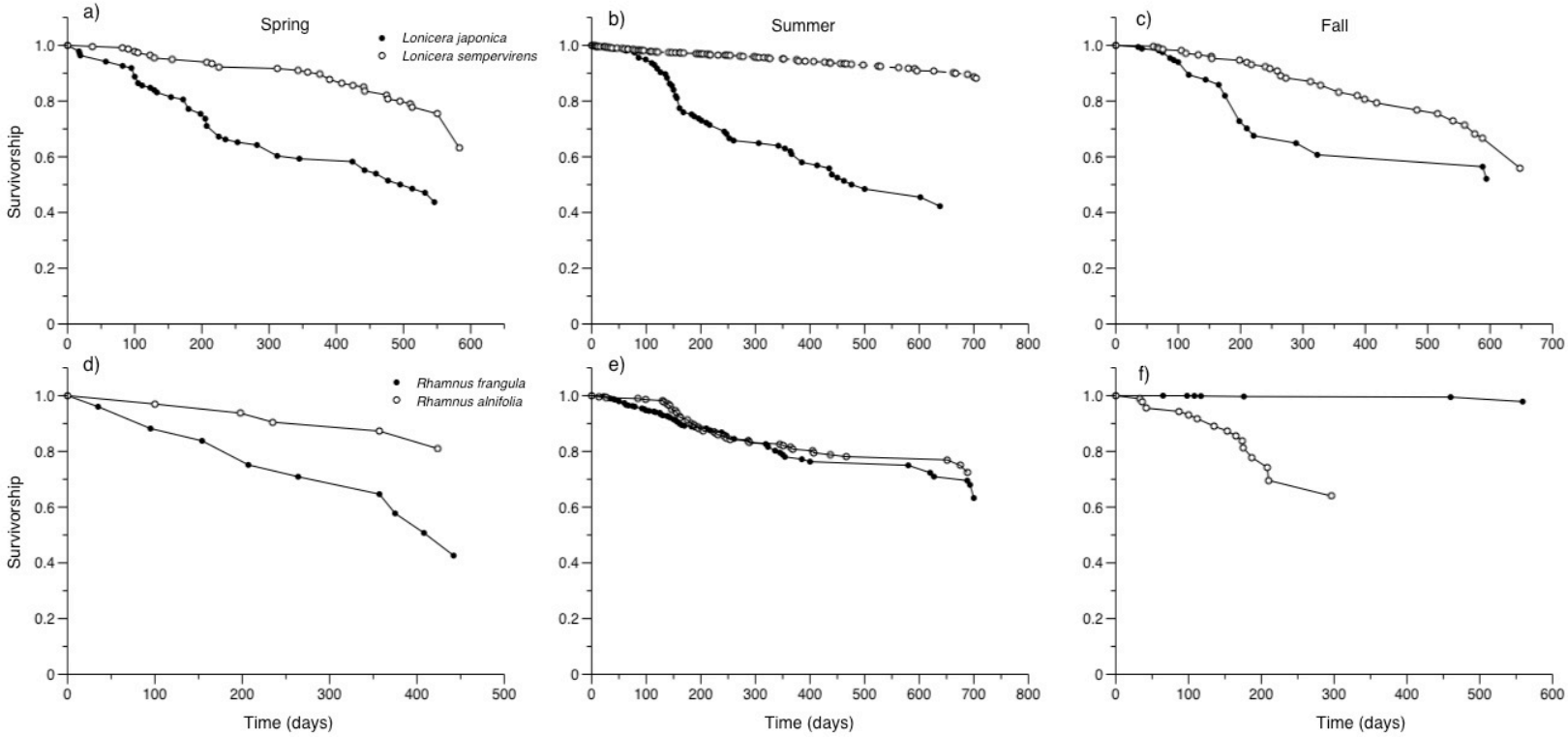

Figure 6 Fine root survivorship of native and non-native congeners (a-c) *L. japonica*, *L. sempervirens* and (d-f) *R. frangula*, *R. alnifolia* over roots born during spring, summer and fall seasons. Data are for all roots observed from July 2010 through October 2011. Open circles (○) indicate non-native congenerics and black circles (●) represent natives.

Relationship between leaf and root production and lifespan

In general, both non-native species exhibited higher aboveground leaf production and lower root production within a single growing season. Average leaf lifespan lasted between 38 days to 120 days. Root and leaf lifespan had the same pattern of shorter-lived tissue in non-native *L. japonica* and longer-lived tissue in native *L. sempervirens*. In *Rhamnus* species, non-native *R. franugla* had shorter-lived roots, although average leaf lifespan was similar between the two species.

Seasonal N uptake

Significant differences were observed with time of uptake, season and the interaction between time and season ($P < 0.0001$, Table 4). The parameters that contributed to significant increases in ^{15}N content were during the six-hour time period ($P < 0.0001$) and the interaction of season and time at six hours ($P < 0.0001$). Uptake was significantly higher during spring than the fall season ($P < 0.0001$, Table 4). For spring and fall, there were no differences in ^{15}N uptake between native and non-native species within either *Rhamnus* and *Lonicera* ($P = 0.233$).

When the analysis was separated by individual season, roots born in the spring had approximately 100 times higher nitrate concentrations after ^{15}N exposure than roots from the fall (Fig. 8, Table 4). During spring uptake, less than 0.01 mmol O_3^- was accumulated in the tissue until hour six, where a significant increase in uptake occurred ($P < 0.0001$). Root initial contact time with nitrate was significant with a comparatively lower level of uptake ($P = 0.042$), so its effect on total uptake was minimal overall. Roots of non-native species had a mean nitrate content of 0.008 ± 0.0007 mmol ^{15}N μg^{-1} root tissue, which was significantly higher than native congenerics ($P = 0.009$). No significant effect of time or native status was found during fall uptake ($P > 0.05$), but there was a lower uptake trend in native plants (Fig. 8a).

Table 4 Results from a mixed-effects model testing native status, season, time and the interaction of season and time across ^{15}N concentrations in roots of *L. japonica*, *L. sempervirens*, *R. frangula*, and *R. alnifolia*.

Factor	DF	Parameter Est	St. Err.	t	$P > \|t\|$
Native status	1				
Non-native		0.00085	0.000501	1.7	0.2332
Time	3				
T(0)		-0.00095	0.000513	-1.85	0.0658
T(3)		-0.00028	0.000495	-0.57	0.5717
T(6)		0.00269	0.00049	5.28	**< 0.0001**
Season	1				
Fall		-0.00199	0.000287	-6.91	**< 0.0001**
Season*Time	3				
Fall*T(0)		0.0008	0.00051	1.56	0.1194
Fall*T(3)		0.00042	0.0005	0.85	0.3953
Fall*T(6)		-0.00228	0.0005	-4.66	**< 0.0001**

Significant differences are in bold. T(x) = time (hours)
^{15}N concentrations are measured in [μg ^{15}N μg root tissue^{-1}]

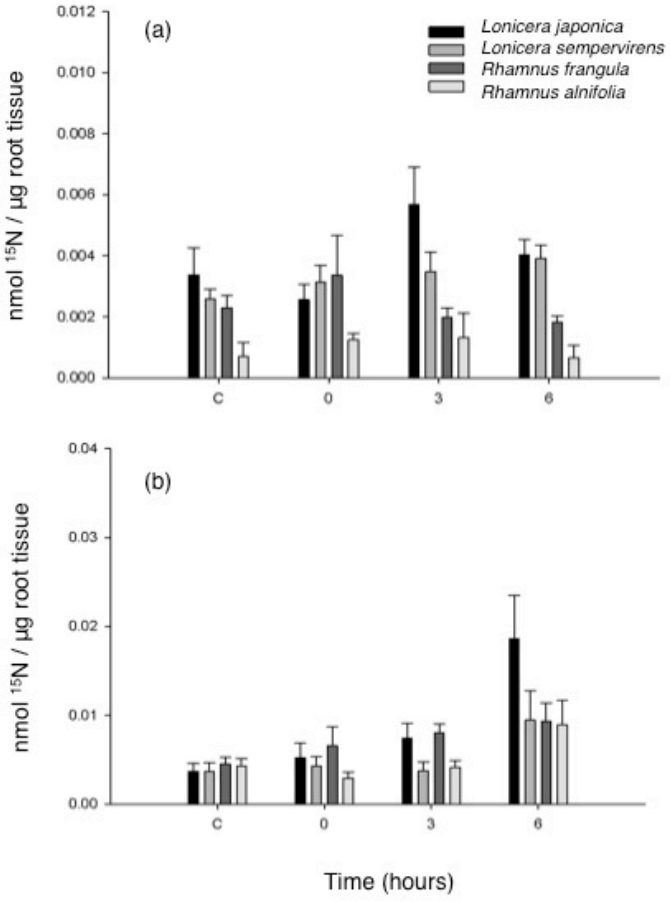

Figure 8 Mean ± SE of ^{15}N (nmol/ug root tissue) accumulated in new first and second order fine roots during (a) fall 2011 and (b) spring 2012 over zero to six hours in 3 hour increments to 1 mmol ^{15}N exposure. C represents the control roots with unlabeled 1 mmol KNO_3 exposure over 6 hours. Species are ordered from left to right: *L. japonica*, *L. sempervirens*, *R. frangula*, *R. alnifolia*. Note the differences in the y-axis scale.

Discussion

Although many studies have explored the relationship of aboveground traits and growth patterns in native and non-native, invasive species (sensu Leishman *et al*. 2007), knowledge of analogous belowground growth dynamics between native and non-native species are still poorly understood, especially at the level of fine roots. Using two congeneric pairs of native and non-native understory plants with vastly contrasting leaf phenology, our results show evidence of links between belowground and aboveground resource-related traits that, together, elucidate growth strategies of non-native species that may underlie their invasiveness.

For *Lonicera* species, non-native *L. japonica* had flushes of root production earlier in the spring and later in the fall, conferring a potential advantage for extended periods of nutrient acquisition. We would expect the timing of root flushes would follow the onset of spring leaf production, for measurements within the same year's root growth, similar to Joslin *et al.* (2001) and Steineker and Wilson (2008), regardless of root and leaf phenology measurements taken across separate years. Potential reasons for root production to follow leaf production could be related to the earlier warming of the atmosphere relative to soil temperatures at the start of the growing season associated with leaf production (Steineker and Wilson 2008) or root growth dependency on aboveground carbon fixation (Fitter *et al*. 1999; Joslin *et al*. 2001). It is also likely that new leaves are produced carbon stored from the previous year's photosynthate, while new roots are produced from partitioned carbon during the current growing season (Steineker and Wilson 2008).

Temporal asynchrony between fine root production and leaf production was seen between both congeneric pairs of species. *Lonicera* vines had the highest periods of fine root production during the spring and fall, while the highest production of leaves occurred during the

summer. In *Rhamnus* shrubs, fine root production was highest during the summer, while leaf production was greatest during the spring. This pattern fit the hypothesis proposed Pregitzer *et al*. (2000) that species partition the growth of above and belowground tissues since foliage and fine roots may be competitive sinks for C. The differences in timing between fine root and leaf production in *Lonicera* and *Rhamnus* is most likely due to growth form. Since vines, unlike shrubs, do not require the same amount of mechanical support for vertical growth, they can initially allocate more to root production rather than increasing stem thickness after initial leaf production, as well as allocate towards summer leaf production and stem length for water transport (Gartner 1991; Isnard and Silk 2009).

Non-native *L. japonica* had lower overall fine root biomass compared with native *L. sempervirens* (Fig. 3, Fig. 4a). However, biomass alone in *Lonicera* vines may not be a suitable indicator for the advantage of non-natives over native species. Schierenbeck *et al.* (1994) found that native *L. sempervirens* had higher biomass accumulation than *L. japonica* in the absence of herbivores, suggesting that annual carbon gain, as an independent factor, does not confer an advantage for this species. In the presence of herbivores, *L. japonica* showed a compensatory response of increased aboveground growth (Schierenbeck *et al.* 1994). If similar to other non-native species that exhibit a compensatory response to aboveground herbivory, defoliation events may decrease root and total biomass, yet increase N uptake from roots and N remobilization from leaves to compensate for increased N demand, leading to short-term growth advantages (Thomas *et al.* 2007).

In addition to differences in fine root production, we also found a strong difference in fine root lifespan between *Lonicera* species ($P < 0.0001$). Unlike Withington *et al*. (2006) who found no correlation of aboveground and belowground traits among 11 temperate tree species,

we found a corresponding pattern in leaf and fine root life span, with non-native *L. japonica* exhibiting significantly shorter fine root life span and leaf lifespan compared with native *L. sempervirens*. Seasonally, fine roots exhibited increasing lifespan from spring to fall in *L. japonica* and life span was variable in *L. sempervirens*, though lowest in the spring. This finding was consistent with other studies of root dynamics in temperate forests, where fine root production and turnover is highest during warmer seasons, perhaps due to increased soil nutrient availability from higher rates of soil microbial decomposition and high photosynthetic and respiration rates that can decrease tissue construction costs and increase fine root turnover, lowering fine root lifespan (Wells and Eissenstat 2001; Tierney *et al*. 2003; Chen and Brassard 2013). However, our results did not corroborate our hypothesis of correspondingly higher tissue production and turnover throughout the fall as non-natives continued extended leaf production. The shorter root life span across seasons in non-native *L. japonica* suggests higher turnover with important implications for more effective resource capture, with the age of roots having a central role in the time period roots are physically active (Volder *et al.* 2005, 2009). The capacity for seasonal fine root N acquisition in individual fine roots of *L. japonica* was demonstrated through higher ^{15}N uptake (Fig. 8b), though fall uptake did not suggest extended seasonal N uptake to support extended leaf phenology.

In contrast to *Lonicera* species, we found no differences between *Rhamnus* species in total and seasonal root production or root mortality to support our hypothesis of a link between extended leaf phenology and fine root growth, despite strong contrasts in aboveground phenology and known differences in growth rate (Fig.1, Ruiz-Robleto and Villar 2005). We did find, however, that native *R. alnifolia* differed from non-native *R. frangula* in the timing of beginning seasonal root growth and earlier leaf drop. For understory species, early leaf

production before canopy closure followed by allocation of photosynthates belowground may be critical to periods of low light during canopy closure and dormant winter months (Walters and Reich 1999). Additionally, in accordance with our hypothesis, native *R. alnifolia* exhibited significantly longer root survivorship than non-native *R. frangula* (Table 2), similar to *Lonicera* tissue longevity, supporting the association between fine root tissue longevity, tissue N concentration, growth rate, and invasive potential (Comas and Eissenstat 2004; Ruiz-Robleto and Villar 2005; Herron *et al*. 2007; Osunkoya *et al*. 2010; Dawson *et al.* 2011). Although no distinct pattern was found in seasonal root life span between species, the small number of uncensored roots during spring and fall seasons may have been a contributing factor to the results, warranting a longer period of observation. While not significant, there was a trend in greater spring N uptake in fine roots of *R. frangula* compared with *R. alnifolia*, suggesting a higher capacity for ephemeral resource capture (Fig. 8b).

In our study, we analyzed multiple covariates contributing to the risk of root mortality. One consistent risk of root mortality across all four species was root order, where first order roots were shorter lived than higher second order roots (Table 2). This is in line with results from Guo *et al.* (2008b) that root branching order had the strongest effect on root life span. However, the lack of influence of root diameter on root mortality from both pairs of congeneric species was contrary to the conclusions from Wells and Eissenstat (2001) and McCormack *et al*. (2012) on the strength of root diameter as a predictor of lifespan. The strength of prediction in longevity between root orders may be a result of differences in categorical orders within the root hierarchical structures, while arbitrary classification based on diameter may include multiple orders of roots (Pregitzer *et al*. 2002; Guo *et al.* 2004, 2008a). Likewise, due to the branching

structure of roots, if higher order roots die then more distal roots must also die, making order a more precise way to categorize roots.

We did not measure mycorrhizal colonization, which has been reported by King *et al.* (2002) as a factor increasing root life span. Mycorrhizal colonization of lower order root tips may be associated with increased protection against pathogens and increased nutrient acquisition (Wolfe and Klironomos 2005). While species of both *Rhamnus* and *Lonicera* genera associate with arbuscular mycorrhizal (AM) fungi, the benefits of mycorrhizal colonization in these species have not been studied, though Harner *et al.* (2010) found that AMF foster successful invasions by other non-native plants in riparian habitats.

We demonstrated here that non-native species differed in growth strategies, with non-native *L. japonica* showing higher biomass allocation to aboveground leaf production and native *L. sempervirens* demonstrating the opposite with higher belowground fine root production, along with differential timing of tissue production between *Rhamnus* species. These differences may be in part to resource use strategies employed by non-native species, which typically exhibit disproportionate bias towards aboveground growth under high light conditions (Sanford *et al*. 2003). Higher biomass allocation to aboveground leaf production in non-natives may stem from the need to pre-empt light resources in the understory before canopy closure. Non-native invasive congeners also showed capacity for high root turnover, associated with increased N uptake, paralleling the capacity for fast growing leaf foliage to capture higher amounts of C (Volder 2005; Osunkoya *et al*. 2010). Future experiments addressing same-year resource partitioning to above- and belowground tissues may provide further insight into whole-plant strategies for successful resource capture strategies.

Works Cited

Allison P.D. (2010) *Survival analysis using the SAS system: a practical guide*. Second edition. Cary, NC, USA. SAS Institute.

Allison S.D. and Vitousek P.M. (2004) Rapid nutrient cycling in leaf litter from non-native plants in Hawai'i. *Oecologia*. 141: 612-619.

Brassard B.W., Chen H.Y.H., and Bergeron Y. (2009) Influence of environmental variability on root dynamics in northern forests. *Critical Reviews in Plant Science*. 28: 179-197.

Burke M.K. and Raynal D.J. (1994) Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant and Soil*. 162: 135-146.

Burton A.J., Pregitzer K.S., and Hendrick R.L. (2000) Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. *Oecologia*. 125: 389-399.

Burton A.J., Jarvey J.C., Jarvi M.P., *et al*. (2012) Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Global Change Biology*. 18: 258-266.

Chen H.Y.H. and Brassard B.W. (2013) Intrinsic and extrinsic controls of fine root life span. *Critical Reviews in Plant Sciences*. 32: 151-161.

Comas L.H. and Eissenstat D.M. (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Functional Ecology*. 18: 388-397.

Cox D. (1972) Regression models and life tables. *Journal of the Royal Statistical Society*. 34: 187-220.

Davis M.A., Grime J.P., and Thompson K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*. 88: 528-534.

Dawson W., Fischer M., and van Kleunen M. (2011) The maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. *Global Biology and Biogeography*. 20: 299-306.

Drenovsky R.E., Grewell B.J., D'Antonio C.M., *et al.* (2012) A functional trait perspective on plant invasion. *Annals of Botany*. 110: 141-153.

Ehrenfeld J.G., Kourtev P., and Huang W. (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. *Ecological Applications*. 11: 1287-1300.

Ehrenfeld J.G. (2003) Effects of exotic plant invasions on soil nutrient cycling processes. *Ecosystems*. 6: 503-523.

Eissenstat D.M. and Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research.* 27: 1-60.

Fitter A.H. (1987) An architectural approach to the comparative ecology of plant root systems. *New Phytologist*. 106: 61-77.

Fitter A.H., Self G.K., Brown T.K., *et al*. (1999) Root production and turnover in an upland grassland subjected to artificial soil warming respond to radiation flux and nutrients, not temperature. *Oecologia*. 120: 575-581.

Fridley J. (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. *Nature*. 485: 359-362.

Funk J.L. and Vitousek P.M. (2007) Resource-use efficiency and plant invasions in low-resource systems. *Nature*. 446: 1079-1081.

Funk J.L. (2008) Differences in plasticity between non-native and native plants from a low resource environment. *Journal of Ecology*. 96: 1162-1173.

Gartner B.L. (1991) Structural stability and architecture of vines vs. shrubs of poison oak, *Toxicodendron diversilobum*. *Ecology*. 72: 2005-2015.

Guo D., Xia M., Wei X., *et al*. (2008a) Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist*. 180: 673-683.

Guo D., Mitchell R.J., Withington J.M., *et al*. (2008b) Endogenous and exogenous controls of roots life span, mortality and nitrogen flux in a longleaf pine forest: foot branch order predominates. *Journal of Ecology*. 96: 737-745.

Heberling J.M and Fridley J.D. (2013) Resource-use strategies of native and invasive plants in Eastern North American forests. *New Phytologist*, in press.

Hendrick R.L. and Pregitzer K.S. (1993) The dynamics of fine root length, biomass, and nitrogen content in two northern hardwood ecosystems. *Canadian Journal of Forest Research*. 23: 2507-2520.

Hendrick R.L. and Pregitzer K.S. (1997) The relationship between fine root demography and the soil environment in northern hardwood forests. *Ecoscience*. 4: 99-105.

Herron P.M., Martin C.T., Latimer A.M., and Leicht-Young S.A. (2007) Invasive plants and their ecological strategies: prediction and explanation of woody plant invasion in New England. *Diversity and Distributions*. 13: 633-644.

Isnard S. and Silk W.K. (2009) Moving with climbing plants from Charles Darwin's time into the 21st century. *American Journal of Botany*. 96: 1205-1221.

Joslin J.D., Wolfe M.H., and Hanson P.J. (2001) Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil*. 228: 201-212.

Kattge J., Diaz S., Lavorel S., *et al*. 2011. TRY – a global database of plant traits. *Global Change Biology*. 17: 2905-2935.

King J.S., Albaugh T.J., Allen H.L., *et al*. (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist*. 154: 389-898.

Kourtev P., Ehrenfeld J., Häggblom M., *et al*. (2002) Exotic plant species alter the microbial community structure and function in the soil. *Ecology*. 83: 3152-3166.

Kourtev P.S., Ehrenfeld J.G., and Häggblom M. (2003) Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. *Soil Biology & Biochemistry*. 35: 895-905.

Kurten E.L., Snyder C.P., Iwata T., and Vitousek P.M. (2008) *Morella cerifera* invasion and nitrogen cycling on a lowland Hawaiian lava flow. *Biological Invasions*. 10: 19-24.

Leishman M.R., Haslehurst T., Ares A., and Baruch Z. (2007) Leaf trait relationships of native and non-native plants: community- and global-scale comparisons. *New Phytologist*. 176: 635-643.

Levine J.M., Montserrat V., D'Antonio C.M., Dukes J.S., Griguli K. and Lavorel S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London.* 270: 775-781.

Lyr H. and Hoffman G. (1967) Growth rates and periodicity of tree roots. *In* International Review of Forest Research, Vol. 2. Eds. Romberger JA and Mikola P. Academic Press, New York, pp 181-236.

Martina J.P. and von Ende C.N. (2012) Highly plastic response in morphological and physiological traits to light, soil-N, and moisture in the model non-native plant, *Phalaris arundinacea*. *Environmental and Experimental Botany*. 82: 43-53.

Matzek V. (2012) Trait values, not trait plasticity, best explain non-native species' performance in a changing environment. *PLoS ONE*. 7: e48821.

McCormack M.L., Adams T.S., Smithwick E.A.H., and Eissenstat D.M. (2012) Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist*. 195: 823-831.

Naeem S., Thompson L.J., Lawler S.P., *et al*. (1994) Declining biodiversity can alter the performance of ecosystems. *Nature*. 368: 734-737.

Osunkoya O.O., Bayliss D., Panetta F.D., and Vivian-Smith G. (2010). Leaf trait co-ordination in relation to construction cost, carbon gain and resource-use efficiency in exotic invasive and native woody vine species. *Annals of Botany*. 106: 371-380.

Post E., Forchhammer M.C., Stenseth N.C., and Callaghan T.V. (2001) The timing of life-history events in a changing climate. *Proceedings of the Royal Society of London Series B*. 268: 15-23.

Pregitzer K.S., Laskowski M.J., Burton A.J., *et al*. (1998) Variation in sugar maple root respiration with root diameter and soil depth. *Tree Physiology*. 18: 665-670.

Pregitzer K.S., King J.S., Burton A.J., and Brown S.J. (2000) Responses of tree fine roots to temperature. *New Phytologist*. 147: 105-115.

Pregitzer K.S., DeForest J.L., Burton A.J., *et al*. (2002) Fine root architecture of nine North American trees. *Ecological Monographs*. 72: 293-309.

Pregitzer K.S. (2002) The fine roots of trees – a new perspective. *New Phytologist*. 156: 267-270.

Pysek P. and Richardson D.M. (2007) Traits associated with non-nativeness in alien plants: where do we stand? *Biological Invasions.* 293: 97-125.

Ruiz-Robleto J. and Villar R. (2005) Relative growth rate and biomass allocation in ten woody species with different leaf longevity using phylogentically independent contrasts (PICs). *Plant Biology*. 7: 484-494.

Sanford N.L., Harrington R.A., and Fownes J.H. (2003). Survival and growth of native and alien woody seedlings in open and understory environments. *Forest Ecology and Management*. 183: 377-385.

Schiernbeck K.A., Mack R.N., and Sharitz R.R. (1994) Effects of herbivory on growth and biomass allocation in native and introduced species of *Lonicera*. *Ecology*. 75: 1661-1672.

Steinaker D.F. and Wilson S.D. (2008) Phenology of fine roots and leaves in forest and grassland. *Journal of Ecology*. 96: 1222-1229.

Tecco P.A., Diaz S., Cabido M., and Urcelay C. (2010) Functional traits of alien plants across contrasting climatic and land-use regimes: do aliens join the locals or try harder than them? *Journal of Ecology*. 98: 17-27.

Thomas M.M., Millard P., Watt M.S., *et al*. (2008) The impact of defoliation on nitrogen translocation patterns in the woody invasive plant, *Buddleia davidii*. *Functional Plant Biology*. 35: 462-469.

Tierney G.L., Fahey T.J., Groffman P.M., *et al*. (2003) Environmental control of fine root dynamics in a northern hardwood forest. *Global Change Biology*. 9: 670-679.

Trammell T.L.E., Ralston H.A., Scroggins S.A., and Carreiro M.M. (2012) Foliar production and decomposition rates in urban forests invaded by the exotic non-native shrub, *Lonicera maackii*. *Biological Invasions*. 14: 529-545.

van Kleunen M., Weber E., and Fischer M. (2010) A meta-analysis of trait differences between non-native and non-non-native plant species. *Ecology Letters*. 13: 235-245.

Vitousek P.M. (1988) Diversity and biological invasions of Oceanic Islands. In: Wilson E. O., Peter F. M. (Eds.), Biodiversity. National Academy of Sciences, Washington, DC, pp.181-189.

Vitousek P.M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos*. 57: 7-13.

Volder A., Smart D.R., Bloom A.J. and Eissenstat D.M. (2005) Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytologist*. 165: 493-502.

Volder A,. Anderson L.J., Smart D.R., *et al*. (2009) Estimating nitrogen uptake of individual roots in container-and field-grown plants using a ^{15}N-depletion approach. *Functional Plant Biology*. 36: 621-628.

Walters M.B. and Reich P.B. (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytologist*. 143: 143-154.

Wells C.E. and Eissenstat D.M. (2001) Marked differences in survivorship among apple roots of different diameters. *Ecology*. 82: 882-892.

Withington J.M., Reich P.B., Oleksyn J., and Eissenstat D.M. (2006) Comparisons of structure and life span in roots and leaves among temperate trees. *Ecological Monographs*. 76: 381-397.

Wright I.J., Groom P.K., Lamon B.B., *et al*. (2004) Leaf trait relationships in Australian plant species. *Functional Plant Biology*. 31: 551-558.

Xu C-C., Griffin K.L., and Schuster W.S.F. (2007) Leaf phenology and seasonal variation of photosynthesis of invasive *Berberis thunbergii* (Japanese barberry) and two co-occurring native understory shrubs in a northeastern United States deciduous forest. *Oecologia*. 154: 11-21.

CHAPTER 2

CONTRASTING XYLEM VESSEL CONSTRAITNS ON HYDRAULIC CONDUCTIVITY BETWEEN NATIVE AND NON-NATIVE WOODY UNDERSTORY SPECIES

Abstract

We examined the hydraulic properties of 83 native and non-native woody species common to forests of Eastern North America, including several congeneric groups, representing a range of anatomical wood types. We observed smaller conduit diameters with greater frequency in non-native species, corresponding to lower calculated vulnerability to cavitation index. Non-native species exhibited higher vessel-grouping in metaxylem compared with native species, however, solitary vessels were more prevalent in secondary xylem. Higher frequency of solitary vessels in secondary xylem was related to a lower vulnerability index. We found no relationship between anatomical characteristics of xylem, origin of species and hydraulic conductivity, indicating that non-native species did not exhibit advantageous hydraulic efficiency over native species. Our results confer anatomical advantages for non-native species under the potential for cavitation due to freezing, perhaps permitting extended growing seasons.

Introduction

Wood xylem vessel members constitute the main pathway for water transport over long distances within a plant and are morphologically diverse across species. In addition to the pronounced differences in ring-porous vs. diffuse-porous species, xylem arrangement follows a continuum of organizational levels. Vessel organization and distribution including vessel number and frequency are associated with varying ecological patterns and adaptive growth strategies (Baas 1983). Moreover, correlations between wood anatomy and factors integrating

seasonal water availability that result in functional adaptation may reveal characteristics representing successful plant hydraulic properties (Taneda and Sperry 2008; Jansen *et al*. 2004) that promote a physiological advantage of non-native over native species (Caplan and Yeakley 2010; Pratt and Black 2006).

High relative growth rate (RGR) is common to many introduced non-native woody plants (Dawson *et al*. 2011). Hydraulic architecture is associated with plant growth rate (Brodribb *et al*. 2002; Meinzer *et al*. 2010), with xylem vessel structure and size identified as the main constraints on maximum water transport and thus hydraulic conductivity (Tyree and Ewers 1991; Steppe and Lemeur 2007). Woodrum *et al*. (2003) examined xylem vessel anatomy and hydraulic conductivity of maples (*Acer*) of varying growth rates but few differences in hydraulic conductivity or anatomical xylem vessel characteristics were apparent. Pratt and Black (2006) were also unable to find differences in cavitation resistance or xylem specific conductivity between five pairs of co-occurring native and non-native trees. However, few if any studies on the relationship between vessel conduit anatomy and water transport in native versus non-native species have been performed to date. Yet, trends in species' hydraulic conductivity have been broadly categorized by means of xylem conduit diameter and rate of water flow (efficiency; Tyree 1994), and that there exists a positive relationship between vessel diameter and growth rate.

Plant water-use strategies are often evaluated as functional trade-offs that maximize resource capture and retention rates based on the resident environment (Grime 2001). Non-native species with invasive potential are considered a threat to native plant communities due to more efficient resource-use (Drenovsky *et al*. 2008; Funk and Vitousek 2007; Grotkopp *et al*. 2002) or higher resource use and capture (Caveleri and Sack 2010) such that they effectively out-compete

natives in their introduced range (Drenovsky *et al*. 2012; Davis *et al*. 2000). One way in which non-natives may increase their efficiency is by decreasing the cost of acquiring or using resources. By investing in cheaper structural tissues (Dale and Causton 1992), species lose benefits associated with increased construction cost which, in turn, influences species' hydraulic vulnerability to water stress or freezing (Carlquist 1977). Elucidating the linkage between wood characteristics such as and water transport provides insight into plant hydraulic functional strategies (Taneda and Sperry 2008; Baas 1986).

Embolism vulnerability places a constraint on maximum water transport through a reduction in hydraulic conductance as a consequence of drought and freeze-thaw cycles (Meinzer *et al*. 2001; Pockman and Sperry 1996) and even normal growth conditions (Cochard and Tyree 1990; Sperry *et al*. 1994; McCully *et al*. 1998). Recent studies have emphasized additional vessel characteristics that are potentially important drivers of hydraulic transport and protection against embolism formation, including inter-vessel pit structure and size as a bottleneck to air-seeding (Lens *et al*. 2010; Christman *et al*. 2009; Choat *et al*. 2008; Jansen *et al*. 2004), vessel perforation plate type (openings at the end of vessel elements; Jansen *et al*. 2004), vessel wall thickness (Hacke *et al*. 2001), and vessel connectivity (Loepfe *et al*. 2007; Lens *et al*. 2011). Comparative studies that investigate vessel characteristics over a wide survey of plant species are limited (but see McCulloh *et al*. 2010; Jansen *et al*. 2004).

Here we examine the hydraulic properties of 83 native and non-native woody species common to forests of Eastern North America, including several congeneric groups, which represent a range of anatomical wood types. Broad surveys containing a large number of species have the potential to reveal functional classification syndromes that relate to plant productivity. Our goal was to compare relationships between hydraulic conductance and xylem vessel

vulnerability by stem vascular structure and arrangement between non-native and native woody species. We examined relationships between stem xylem vessel anatomical arrangement, xylem vessel types, stem specific hydraulic conductivity, and cavitation vulnerability among native and non-native woody understory species. Specifically, we tested the hypothesis that non-native understory species have greater capacity for water transport than native understory species.

Materials and Methods

Plant Material and Growing Conditions

Stem material was harvested from mature plants in a common garden comprised of a homogenous, clay loam soil in Syracuse, NY, USA (43°03' N, 76°09' W), representing a range of native and non-native woody species including several common genera (Table 1). Each species was grown in three replicate blocks, each under 80% shade cloth during the growing season (late May – late October) to simulate deciduous forest conditions. Species were obtained from natural areas in central New York when possible; those species not available in our region were sourced from commercial growers located in the northern U.S. Plants were pruned occasionally over the 5-yr period preceding stem harvest but not fertilized or watered, and summer wilting was not observed.

Table 1 Woody shrub species list tested and attributes. Mean vessel traits, vessel diameter (VD), vessel frequency (VF), vulnerability index (VI), and stem hydraulic conductivity (Ks), for each species.

Species	Family	Invasive status	Porosity type	Perforation Plate Type	VD (μm)	VF (N/m m^2)	VI (VD/VF)	K_s (10^{-3} ks s MPa^{-1} m^{-3})
Acer negundo	Sapindaceae	native	diffuse	simple	21.98	42.70	0.51	3.05
Acer pensylvanicum	Sapindaceae	native	diffuse	simple	26.40	18.80	1.40	*
Acer saccharum	Sapindaceae	native	diffuse	simple	20.35	44.90	0.45	*
Berberis canadensis	Berberidaceae	native	semi-ring	simple	12.60	80.27	0.16	*
Berberis koreana	Berberidaceae	non-native	ring	simple	16.77	62.90	0.27	8.69
Berberis thunbergii v. atropurpurea	Berberidaceae	non-native	semi-ring	simple	12.37	113.70	0.11	3.23
Berberis vulgaris	Berberidaceae	non-native	ring	simple	13.02	123.53	0.11	1.45
Calycanthus floridus	Calycanthaceae	native	semi-ring	simple	26.00	35.70	0.73	*
Celastrus orbiculatus	Celastraceae	non-native	ring	simple	22.11	32.10	0.69	4.52
Celastrus scandens 'diana'	Celastraceae	native	ring	simple	17.7	57.67	0.31	4.97
Cephalanthus occidentalis	Rubiaceae	native	ring	simple	16.29	52.27	0.31	2.36
Chionanthus virginicus	Oleaceae	native	ring	simple	20.6	24.40	0.84	3.96
Cornus alternifolia	Cornaceae	native	diffuse	scalariform	26.43	31.20	0.85	1.12
Cornus amomum	Cornaceae	native	diffuse	scalariform	28.49	30.80	0.92	3.02
Cornus florida	Cornaceae	native	diffuse	scalariform	17.80	32.50	0.55	*
Cornus mas	Cornaceae	non-native	diffuse	scalariform	15.91	28.60	0.56	5.45
Cornus sericea	Cornaceae	native	diffuse	scalariform	23.6	70.60	0.33	6.23
Diervilla lonicera	Caprifoliaceae	native	diffuse	scalariform	25.30	55.20	0.46	0.98
Diervilla rivularis	Caprifoliaceae	native	diffuse	scalariform	22.60	42.40	0.53	*
Dirca palustris	Thymelaeaceae	native	diffuse	simple	15.5	68.20	0.23	0.58
Elaeagnus angustifolia	Elaeagnaceae	non-	diffuse	simple	24.40	19.10	1.28	*

		native						
Elaeagnus commutata	Elaeagnaceae	native	semi-ring	simple	16.50	62.36	0.26	*
	Elaeagnaceae	non-						
Elaeagnus multiflora		native	ring	simple	28.68	35.60	0.81	7.59
	Elaeagnaceae	non-						
Elaeagnus pungens		native	diffuse	simple	13.30	85.40	0.16	*
	Elaeagnaceae	non-						
Elaeagnus umbellata		native	semi-ring	simple	25.80	28.27	0.91	*
	Celastraceae	non-						
Euonymus alatus		native	diffuse	simple	15.86	56.00	0.28	1.29
Euonymus americanus	Celastraceae	native	ring	simple	11.70	142.00	0.08	*
Euonymus atropurpureus	Celastraceae	native	diffuse	simple	17.25	78.67	0.22	6.29
	Celastraceae	non-						
Euonymus bungeanus		native	diffuse	simple	18.18	72.40	0.25	10.1
Euonymus europeaus	Celastraceae	non-						
'atrorubens'		native	semi-ring	simple	15.70	91.47	0.17	*
Euonymus hamiltonianus	Celastraceae	non-						
sieboldianus		native	diffuse	simple	18.88	84.80	0.22	9.08
Euonymus obovatus	Celastraceae	native	semi-ring	simple	10.60	157.58	0.07	*
	Celastraceae	non-						
Euonymus phellomanus		native	diffuse	simple	13.60	104.56	0.13	*
	Rhamnaceae	non-						
Frangula alnus		native	semi-ring	simple	20.8	31.30	0.66	0.25
Frangula caroliniana	Rhamnaceae	native	semi-ring	simple	25.08	32.80	0.76	16.3
Hamamelis virginiana	Hamamelidaceae	native	diffuse	scalariform	21.21	58.00	0.37	8.68
Hydrangea arborescens	Hydrangeaceae	native	semi-ring	scalariform	21.55	51.10	0.42	1.22
Hydrangea paniculata	Hydrangeaceae	non-						
'Floribunda'		native	diffuse	scalariform	19.60	30.20	0.65	*
Hydrangea quercifolia	Hydrangeaceae	native	semi-ring	scalariform	24.37	50.40	0.48	0.69
	Caprifoliaceae	non-						
Kolokowitzia amabilis		native	ring	scalariform	16.46	38.40	0.43	13.6
Lindera benzoin	Lauraceae	native	diffuse	simple	16.21	36.90	0.44	0.24
Lonicera canadensis	Caprifoliaceae	native	diffuse	simple	13.20	83.53	0.16	*

Lonicera fragrantissima	Caprifoliaceae	non-native	semi-ring	simple	14.02	55.20	0.25	0.66
Lonicera fragrantissima	Caprifoliaceae	non-native	diffuse	simple	13.60	64.70	0.21	*
Lonicera hirsuta	Caprifoliaceae	native	diffuse	simple	17.80	87.60	0.20	*
Lonicera involucrata var involucrata	Caprifoliaceae	native	semi-ring	simple	15.12	93.53	0.16	1.70
Lonicera japonica 'halliana'	Caprifoliaceae	non-native	diffuse	simple	24.70	27.60	0.89	*
Lonicera morrowii	Caprifoliaceae	non-native	semi-ring	simple	15.04	51.80	0.29	0.63
Lonicera nitida	Caprifoliaceae	non-native	semi-ring	simple	11.77	120.40	0.1	8.79
Lonicera oblongifolia	Caprifoliaceae	native	semi-ring	simple	14.10	56.90	0.25	*
Lonicera periclymenum 'GS Thomas'	Caprifoliaceae	non-native	diffuse	simple	24.80	44.07	0.56	*
Lonicera pileata	Caprifoliaceae	non-native	semi-ring	simple	12.30	116.00	0.11	*
Lonicera ruprechtiana	Caprifoliaceae	non-native	semi-ring	simple	16.06	50.50	0.32	4.21
Lonicera sempervirens	Caprifoliaceae	native	semi-ring	simple	24.92	36.00	0.69	2.47
Lonicera standishii	Caprifoliaceae	non-native	diffuse	simple	12.30	80.57	0.15	*
Lonicera tatarica	Caprifoliaceae	non-native	semi-ring	simple	15.57	74.87	0.21	3.52
Lonicera villosa var villosa	Caprifoliaceae	native	semi-ring	simple	14.10	57.50	0.25	*
Lonicera x bella	Caprifoliaceae	non-native	diffuse	simple	16.50	64.00	0.26	*
Lonicera xylosteum	Caprifoliaceae	non-native	semi-ring	simple	15.46	69.95	0.22	1.40
Ptelea trifoliata	Rutaceae	native	ring	simple	23.99	12.44	1.93	0.21
Rhamnus alnifolia	Rhamnaceae	native	semi-ring	simple	18.90	37.80	0.50	*
Rhamnus cathartica	Rhamnaceae	non-	semi-ring	simple	14.40	58.80	0.24	*

		native						
	Rhamnaceae	non-						
Rhamnus davurica		native	ring	simple	14.23	84.20	0.17	11.6
	Grossulariaceae	non-						
Ribes rubrum 'cherry'		native	diffuse	scalariform	16.2	60.00	0.27	1.56
	Rosaceae	non-						
Rosa multiflora		native	diffuse	simple	16.09	54.17	0.3	4.65
Rosa palustris	Rosaceae	native	semi-ring	simple	19.90	48.20	0.41	*
Sambucus nigra ssp	Adoxaceae							
canadensis		native	diffuse	simple	27.52	28.60	0.96	0.17
Sambucus racemosa	Adoxaceae	native	ring	simple	26.45	42.00	0.63	3.46
Shepherdia argentea	Elaeagnaceae	native	ring	simple	18.55	43.00	0.43	4.39
Shepherdia canadensis	Elaeagnaceae	native	diffuse	simple	16.49	85.50	0.19	2.27
		non-						
Stephanandra incisa 'crispa'	Rosaceae	native	diffuse	simple	16.50	34.40	0.48	*
Viburnum acerifolium	Adoxaceae	native	diffuse	scalariform	14.50	68.80	0.21	*
Viburnum dentatum	Adoxaceae	native	diffuse	scalariform	22.4	38.80	0.58	4.45
	Adoxaceae	non-						
Viburnum dilatatum		native	diffuse	scalariform	20.06	51.53	0.39	2.32
Viburnum edule	Adoxaceae	native	diffuse	scalariform	27.22	50.20	0.54	3.93
	Adoxaceae	non-						
Viburnum lantana		native	diffuse	scalariform	20.54	58.10	0.35	1.16
Viburnum lentago	Adoxaceae	native	diffuse	scalariform	22.06	51.00	0.43	2.70
Viburnum nudum ssp	Adoxaceae							
cassanoides		native	semi-ring	scalariform	15.90	65.80	0.24	*
	Adoxaceae	non-						
Viburnum opulus		native	diffuse	scalariform	22.10	63.50	0.35	*
Viburnum opulus var	Adoxaceae							
americana		native	diffuse	scalariform	19.26	71.50	0.27	4.21
Viburnum prunifolium	Adoxaceae	native	diffuse	scalariform	15.00	46.70	0.32	*
Viburnum rafanesquianum	Adoxaceae	native	diffuse	scalariform	16.76	66.60	0.25	26.3
		non-						
Viburnum setigerum	Adoxaceae	native	diffuse	scalariform	20.20	45.60	0.44	*

*Denotes species without high pressure flow meter (HPFM) data

Hydraulic conductivity measurements

Three terminal branch stems containing one year's growth were randomly sampled from individuals of each species from each of three replicate blocks in November 2011. Stems were kept moist in damp paper towels in a cooler approximately two hours prior to taking measurements in a temperature-controlled room at 25°C, equal to the temperature of the high pressure flow meter (HPFM) (Dynamax Inc., Houston, TX, USA). Diameter and stem length were recorded, and the cortex was shaved from the proximal end of stems prior to attachment to a HPFM.

Stem hydraulic conductance (K_h) was measured directly with the HPFM, using methods described by Tyree *et al*. (1995). Each measurement was recorded approximately 30 seconds after stems had become completely saturated with water. Conductance (K_h, kg s MPa^{-1}), the inverse of resistance, was measured by the force of pressurized water through the stem (P) (MPa m^{-1}) and the rate of water flow (F) (Kg s^{-1}). Conductance was calculated as the slope of the regression plot F vs. P:

$K_h = dF/dP$

Stem hydraulic measurements were conducted using quasi-steady state, where F and P are approximately constant (Tyree *et al*. 1993, 1995) and specific stem hydraulic conductivity (K_s) was calculated using stem length and diameter cross-sectional area (kg s MPa^{-1} mm^{-3}).

Anatomical measurements

Two to three stem segments used for hydraulic conductivity were used for anatomical sectioning following conductivity measurements. In addition, three supplemental stem segments collected from the same plants in November 2010 were also sectioned for anatomical measurements. One cm long segments in random locations were removed from the stem,

immediately preserved in formalin-acetic acid-alcohol solution (FAA), and stored at room temperature until the embedding process. Samples were dehydrated in a series of ethanol-tertiary butanol (TBA) dilutions before infiltration with pure TBA (Ruzin 1999).

Stem anatomical samples were embedded in successive changes of Paraplast Plus embedding medium (McCormick Scientific, Saint Louis, Missouri, USA) in a 60°C drying oven for two days. Samples were embedded in a final paraffin change hardened with 15% (v:v) paramount (Fischer Scientific, Fair Lawn, New Jersey, USA). Transverse cross-sections were cut at 20 μm increments using a rotary microtome (HM 355S, Microm International GmbH, Walldorf, Germany). Cross sections were stained with saffranin-O [1% (w:v) in 50% ethanol] and counter-stained using fast-green [0.1% (w:v) in 1:1 absolute ethanol and clove oil] in a series with histo-clear (National Diagnostics, Atlanta, Georgia, USA) to remove paraffin.

Five images per stem were randomly selected for imaging using 20x magnification with a compound light microscope with a fixed camera attachment (Olympus Imaging Corp., Tokyo, Japan). Images representing 0.77 mm^2 cross sectional area were first processed through Photoshop (CS5; Adobe Systems Inc., Mountain View, CA, USA) to select and fill each individual vessel, and then analyzed for xylem vessel lumen cross sectional area (VA) using the image-analysis software Image J (National Institute of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ji/). Vessel area was converted to diameter (VD) assuming circularity of vessels. Conduit-containing sections of each image were then randomly cropped to 0.09mm^2 to represent the xylem area of the smallest stem to obtain vessel frequency (VF) over a uniform area for all species. Vessel vulnerability during water stress was determined using methods from Carlquist (1977) where vulnerability index (VI) = VF/VD. To parse seasonal differences in vessel traits and vulnerability, three equal concentric rings were overlaid on the cross section to

delineate the first, middle, and last rows of vessels to represent the seasonal transition in vessel development from early to late season. Vessel measurements within the three rings were analyzed using the same method as above. Xylem vessel diameter of all 84 species was classified into 7 classes from < 10 μm to > 35 μm at 5 μm intervals. The frequency of each class was estimated.

Vessel groupings were classified into four categories in both metaxylem, the primary xylem that differentiates after the protoxylem and is characterized by broader vessels, and secondary xylem, i.e. the categories of 2, 3, 4, and more than 5 vessels grouped together. The amount of vessel groupings in each classification was counted. For each classification we then calculated grouped vessel percentage (the percentage of the number of vessels grouped relative to the total number of vessels), vessel grouping index (mean number of vessels per vessel grouping), and the percentage of solitary vessels relative to the total number of vessels.

Statistical methods

Differences in the distribution of vessel class frequency were assessed between native and non-native understory species using the Chi-square test. Differences in factors predicting conductivity, vessel traits and vulnerability index were tested using mixed-effect models to control for variability from genus classification. A bivariate regression analysis tested for significance in relationships between conductivity and vessel traits, where conductivity and vulnerability index were log-transformed to improve the assumption of normality. A linear 90th quantile regression was performed using the "quantreg" package from R (v. 2.13.1) to estimate the slope of the 'packing limit' of vessels, representing the upper limit of the number of vessels that can fit in a given area based on size. Forty-nine of the 83 species, those with conductivity measurements, were analyzed for significance in VD over three distinct rows of xylem vessels,

representing three different periods of annual wood formation (the first, middle and last row of selected vessels). A linear mixed model was constructed to determine how time influences vessel sizes between porosity type, origin, and perforation plate type. Genus was included as a random variable, and vessel row was used as an interaction term for time across effects. Vessel grouping in metaxylem was only compared between non-native and native species that had metaxylem using the Mann-Whitney U-test. Each classification of vessel grouping in secondary xylem was compared respectively between non-native and native species using one-way analysis of variance (ANOVA). Data were tested for normality and homogeneity to determine if it matched the assumptions of ANOVA. A mixed effects model was used to test for significant predictors of water use efficiency (WUE), using origin and differing light levels across 38 of the 83 species. Species was used as random factor, due to non-independence of repeated measures. Tukey's HSD post-hoc analysis was performed to distinguish differences between light levels. The relationships between vulnerability index and vessel groupings traits were examined individually by linear regression. All analyses excluding quantile regression were performed using JMP (SAS Institute Inc., Cary, NC, v. 10.0).

Results

Of the 83 species studied, porosity type did not differ with origin ($P = 0.970$). When species were separated by porosity type, 55.3% of native species had a diffuse porous xylem ring structure, 29.8% had a semi-ring, and 14.9% had a ring porous vessel. Non-native species had a distribution comprised of 52.6% diffuse porous, 31.6% semi-ring porous, and 15.8% ring porous. However, contributions of some overrepresented genera drove much of the porosity type distribution. When accounting for genus, members of *Viburnum* and *Lonicera* comprised

approximately 37% of the total individuals, with most species within a genus sharing similar perforation plate type and porosity type traits (Table 1).

Vessel size distribution

The distribution of vessel class frequency differed between native and non-native species ($P < 0.0001$, Fig. 1). Vessels with diameters less than 20 μm appeared more frequently in non-native species than native species. The diameter of more than 50% of the vessels in non-native species ranged from 10 to 20 μm, in which 33.9% of the vessels had diameters between 10 and 15 μm. Frequency of the vessels with diameters more than 20 μm was higher in native species than non-native species. The diameter of more than 70% of the vessels in native species ranged uniformly from 10 to 25 μm, with the 15 to 20 μm interval class having the highest vessel frequency, 26.1%.

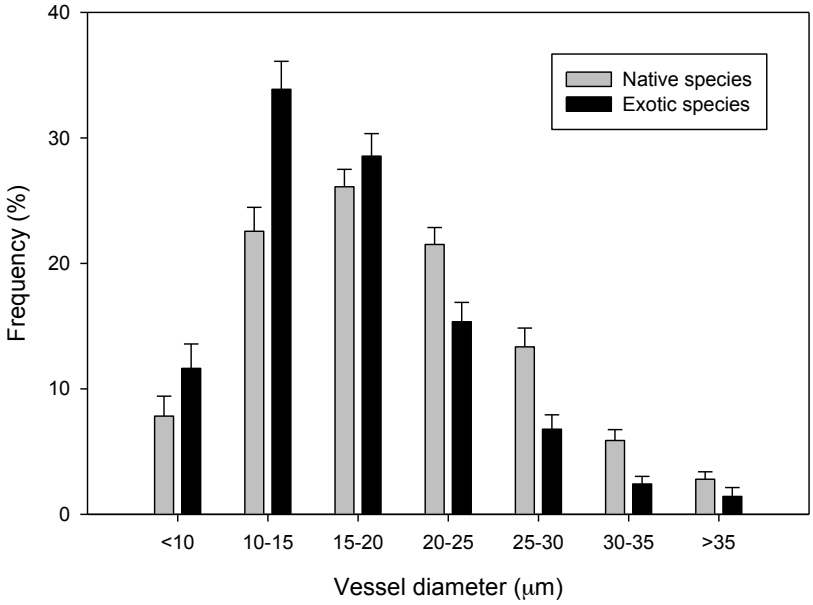

Figure 1: Frequency distribution of xylem vessel diameter (μm) (± 1 SE) for 52 native (gray bars) and non-native (black bars) woody shrub species.

Hydraulic conductivity (K_s)

The mixed-effects model did not show any effects from porosity, perforation plate or status as native or non-native on hydraulic conductivity ($P > 0.10$).

Vessel traits and Vulnerability Index

No differences in VD, VF or VI were found across porosity type, origin or perforation plate type ($P > 0.10$, Table 2). Despite an insignificant difference, non-native species had lower log VI (-0.978 ± 0.167) compared with native species (-0.880 ± 0.141), which was the result of a lower but significant VD of non-native species at $\alpha = 0.10$ ($P = 0.069$). Overall, non-native species had smaller vessels and an overall lower vulnerability index.

Relationships between VD, VF, VI, Vessel area and K_s

A positive relationship was observed between log-transformed vessel area and log-transformed hydraulic conductivity ($P = 0.033$). This indicates faster water movement in species with a higher number of vessels per xylem area than those with less vessel area per unit xylem area (Fig. 3). There was no correlation between K_s and VI ($P = 0.668$). When VI was divided into the individual components of vessel diameter (VD) and vessel frequency (VF) to test for a relationship with K_s, no relationship was found for either VD ($P = 0.3565$) or VF ($P = 0.380$).

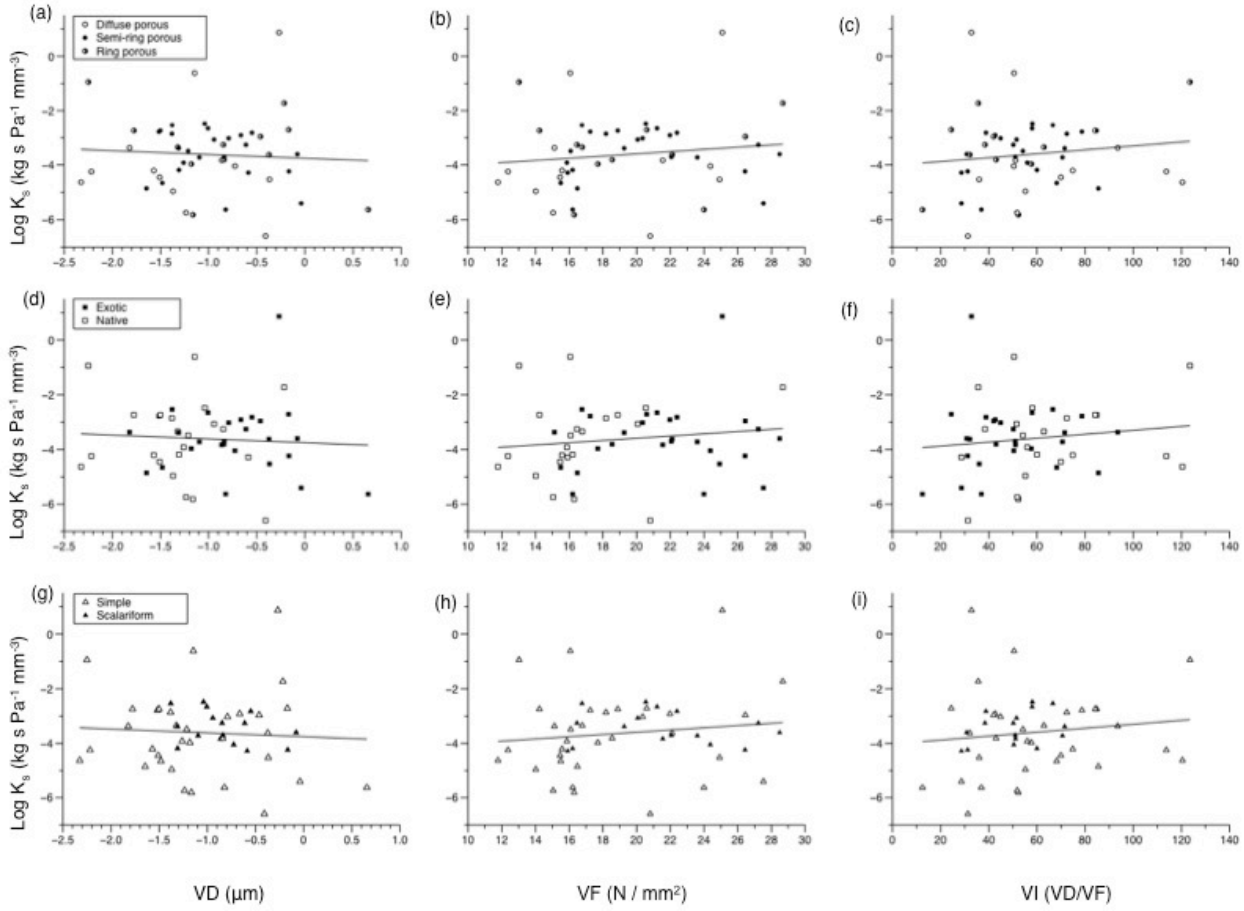

Figure 2 Bivariate fit of vessel traits: diameter (VD), frequency (VF) and vulnerability index (VI) versus the log values of stem conductivity (K_s). (a-c) show VD, VF, and VI by porosity, (d-f) by origin, and (g-i) by perforation plate type. All relationships with log(K_s) were not correlated ($P > 0.10$).

Table 2 Mean (± SE) vessel diameter (VD), vessel frequency (VF), and vulnerability index (VI) of a mixed effects model. F- and P-values are shown.

Factor	VD (μm)	VF (N / mm^2)	VI
Porosity			
Diffuse	19.98 (0.93)	47.95 (5.90)	- 0.82 (0.15)
Semi-ring	18.43 (1.19)	57.68 (7.30)	- 1.06 (0.17)
Ring	19.95 (1.46)	53.86 (8.81)	- 0.85 (0.21)
F-value	*0.99*	*1.08*	*1.18*
P-value	*0.38*	*0.34*	*0.93*
Origin			
Native	20.15 (0.92)	53.54 (5.83)	- 0.90 (0.14)
Non-native	18.76 (1.06)	52.78 (6.60)	- 0.93 (0.16)
F-value	*2.05*	*0.01*	*0.09*
P-value	*0.16*	*0.89*	*0.76*
Perforation Plate Type			
Simple	18.73 (0.88)	54.27 (5.71)	- 0.93 (0.14)
Scalariform	20.18 (1.45)	52.06 (9.36)	- 0.90 (0.23)
F-value	*0.75*	*0.04*	*0.008*
P-value	*0.40*	*0.84*	*0.93*

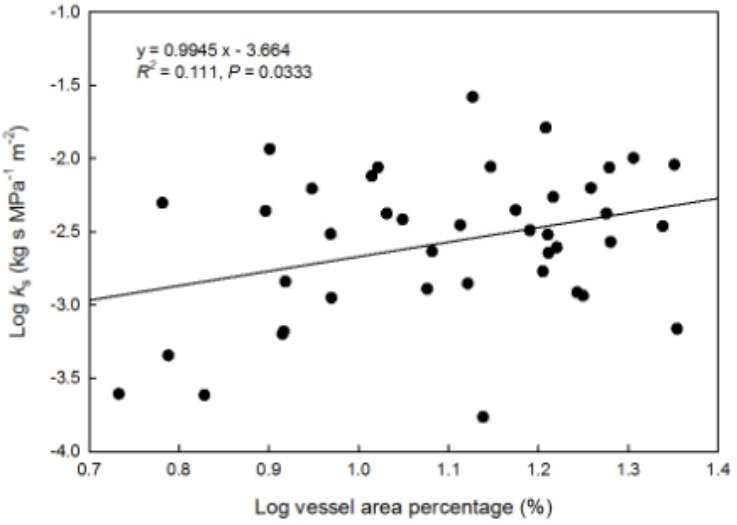

Figure 3 Relationship between log(vessel area) as a percentage of the total viewing area versus hydraulic conductivity (kg s MPa^{-1} m^{-2}). Solid line represents the line of best fit ($\log[K_s] = 0.095(\log[\text{vessel area }\%]) + 3.66$). Points represent species with available HPFM data.

Vessel packing

Overall, an inverse relationship between vessel diameter (VD) and vessel frequency (VF) was found for both native and non-native species (Fig. 4), indicating the larger the conduit diameter, the fewer number of conduits that can occupy a given area of wood. The slope of the constraint line for native species (-6.382) was steeper than the slope for non-native species (-4.909).

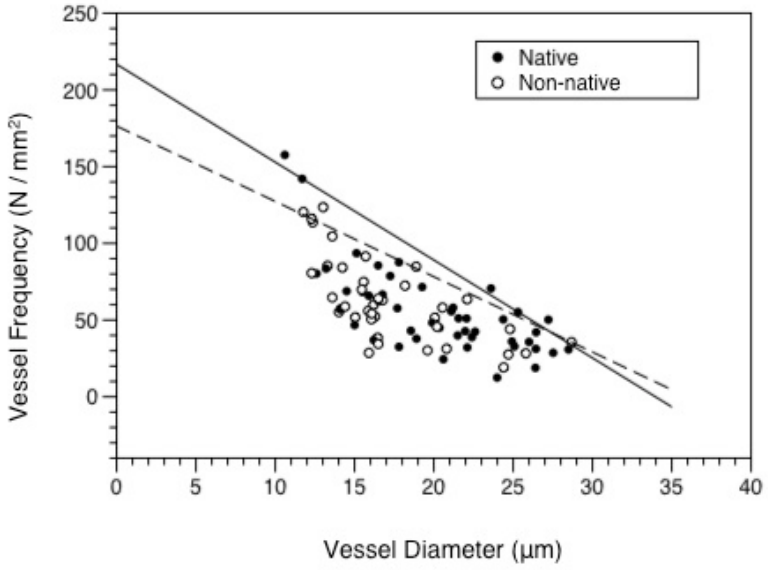

Figure 4 Constraint relationship of vessel frequency (VF, N/mm^2) versus diameter (VD, µm) for native and non-native species. Solid line represents the fit of the 90th quantile of native species (VF = 216.674 – 6.382*VD), the dashed line for the 90th quantile of non-native species (VF = 176 .386 – 4.909 *VD). Points are shown for origin (Closed circles = native, open circles = non-native).

Timing of formation and influence on vessel size

Genera explained 39% of the total variability within the model ($P < 0.10$), suggesting that variation among genera contributes to differences in VD. Timing of vessel development was a significant factor ($P < 0.001$). In a comparison of vessel rows, the last row of vessels was significantly smaller than that of both the first and middle rows ($P < 0.10$, Table 3). No interaction was detected between timing of vessel development and porosity, perforation plate type, or origin ($P > 0.10$). Only origin had an effect on vessel diameter ($P < 0.001$). Porosity type and perforation plate type had no predictive effect on vessel diameter (Table 2). The variability contributed by vessel row was 16%, compared with 32% for genera. Again, origin was the only significant factor in predicting VD ($P < 0.0001$).

Table 3 The effect of vessel development timing, perforation plate type, porosity, origin, and interactions on vessel diameter (VD, μm). Significant effects are in bold.

Factor	VD (μm)	F	*P*
Vessel row		11.664	**< 0.0001**
First	20.003 (0.904)		
Middle	20.889 (0.904)		
Last	17.215 (0.904)		
Perforation plate type		1.092	0.309
Porosity		1.035	0.364
Perf plate x vessel row		2.065	0.132
Porosity x vessel row		0.648	0.630
Origin		25.823	**< 0.0001**
Native	21.234 (0.839)		
Non-native	17.505 (0.882)		
Origin x vessel row		0.089	0.915

Vessel groupings

Approximately 62% of non-native species had metaxylem, while only 16% of native species had metaxylem. Comparing the vessel groupings in metaxylem of the non-native species to the few native species that had metaxylem, non-natives had a higher number of vessel groupings in the ≥ 5-vessel grouping class ($P = 0.018$, Table 4).

In the secondary xylem, native species had significantly more vessel groupings in the 3- ($P = 0.011$) and 4-vessel grouping classes ($P < 0.0001$). The proportion of vessel groupings to total vessels was also significantly higher in native species, compared with non-natives, in these two categories ($P = 0.0099$ for the 3-vessel grouping class and $P < 0.0001$ for the 4-vessel grouping class). Thus, the vessel grouping index in native species was higher than that in non-native species ($P = 0.027$); in other words, non-native species had a higher proportion of solitary vessels in the secondary xylem than native species ($P = 0.028$).

There was a negative linear relationship between average vessel diameter and proportion of solitary vessels to total xylem vessels (Fig. 5) suggesting that species with smaller vessels tend to have fewer vessel groupings.

There was a decrease in the vulnerability index with increasing percentage of solitary vessels (Fig. 6). Specifically, the vulnerability index decreased with decreasing percentage of vessel groupings in the 2, 3, and 4-vessel grouping classes, but not in ≥5-vessel grouping class (Fig. 7).

Table 4 Vessel grouping in metaxylem and secondary xylem of native and non-native species. Significant P-values for the comparisons are shown in italics; n.s. indicates non-significant difference.

	Native	Non-native	*P - value*
Vessel groupings in metaxylem			
Number of 2-vessel groupings	4.51 (0.60)	4.84 (0.73)	*n.s.*
Number of 3-vessel groupings	3.10 (0.48)	3.06 (0.42)	*n.s.*
Number of 4-vessel groupings	1.71 (0.61)	2.16 (0.29)	*n.s.*
Number of > 5-vessel groupings	1.96 (0.58)	4.27 (0.82)	*0.0184*
2-vessel grouping (%)	21.27 (2.94)	19.02 (2.94)	*n.s.*
3-vessel grouping (%)	16.92 (1.36)	12.08 (1.94)	*n.s.*
4-vessel groping (%)	9.83 (1.28)	7.23 (0.73)	*n.s.*
> 5-vessel grouping (%)	9.69 (2.12)	12.08 (1.22)	*n.s.*
Vessel grouping index	3.14 (0.13)	3.44 (0.14)	*n.s.*
Solitary vessels (%)	13.97 (4.11)	22.80 (3.86)	*n.s.*
Vessel groupings in secondary xylem			
Number of 2-vessel groupings	21.51 (2.07)	17.46 (2.08)	*n.s.*
Number of 3-vessel groupings	4.99 (0.45)	3.29 (0.42)	*0.0114*

Number of 4-vessel groupings	3.30 (0.35)	1.29 (0.23)	*< 0.0001*
Number of > 5-vessel groupings	3.00 (0.44)	2.65 (0.85)	*n.s.*
2-vessel grouping (%)	23.72 (1.72)	19.70 (1.92)	*n.s.*
3-vessel grouping (%)	9.04 (0.87)	5.86 (0.77)	*0.0099*
4-vessel groping (%)	7.46 (0.82)	2.77 (0.43)	*< 0.0001*
> 5-vessel grouping (%)	9.60 (2.02)	5.57 (1.09)	*n.s.*
Vessel grouping index	2.61 (0.06)	2.43 (0.07)	*0.0271*
Total solitary vessels (%)	51.43 (2.98)	60.49 (2.52)	*0.0277*

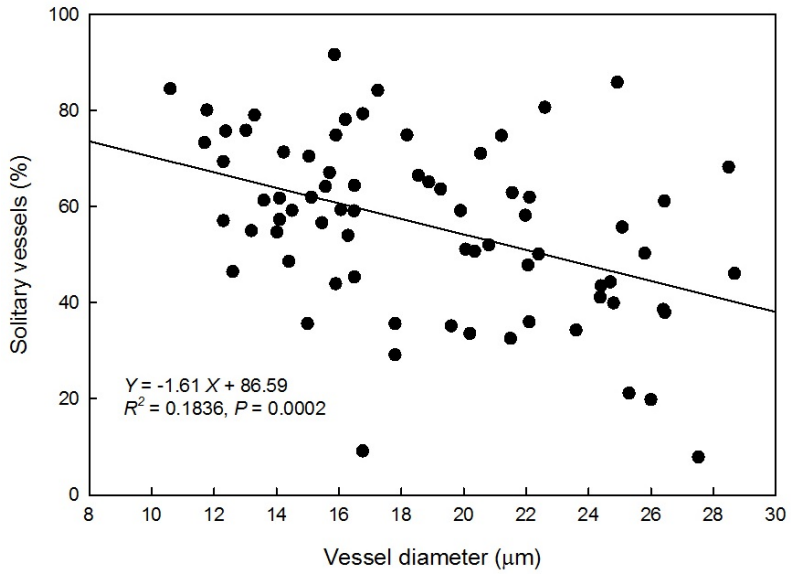

Figure 5 Relationship between vessel diameter (μm) and the proportion of solitary vessels to total vessels in the xylem cross-section of each species. Solid line represents the line of best fit ((percentage of solitary vessels) = - 1.61 (vessel diameter) + 86.59).

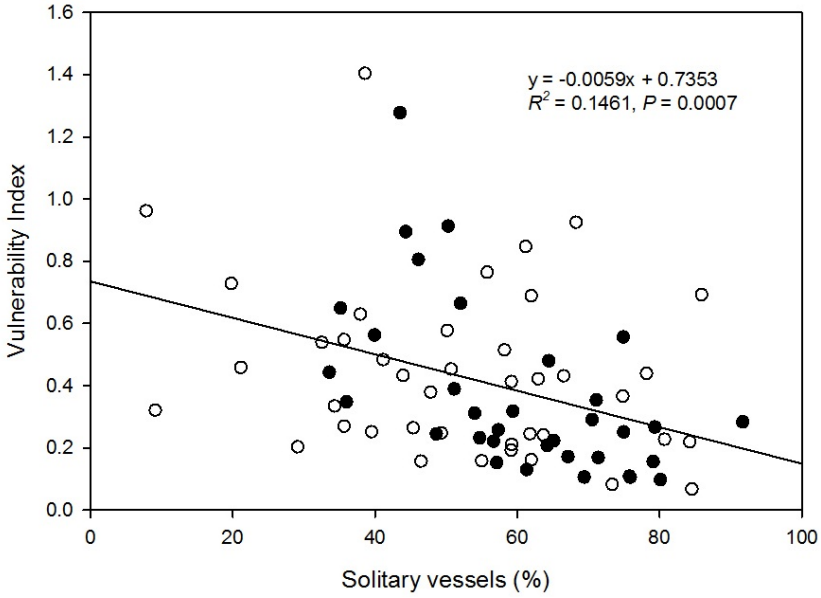

Figure 6 Relationship between vulnerability index and proportion of solitary vessels to total vessels in the xylem cross-section of each species. Filled circles represent native species, and open circles represent native species. Solid line represents the line of best fit ((vulnerability index) = 0.0059 (solitary vessel) + 0.7353).

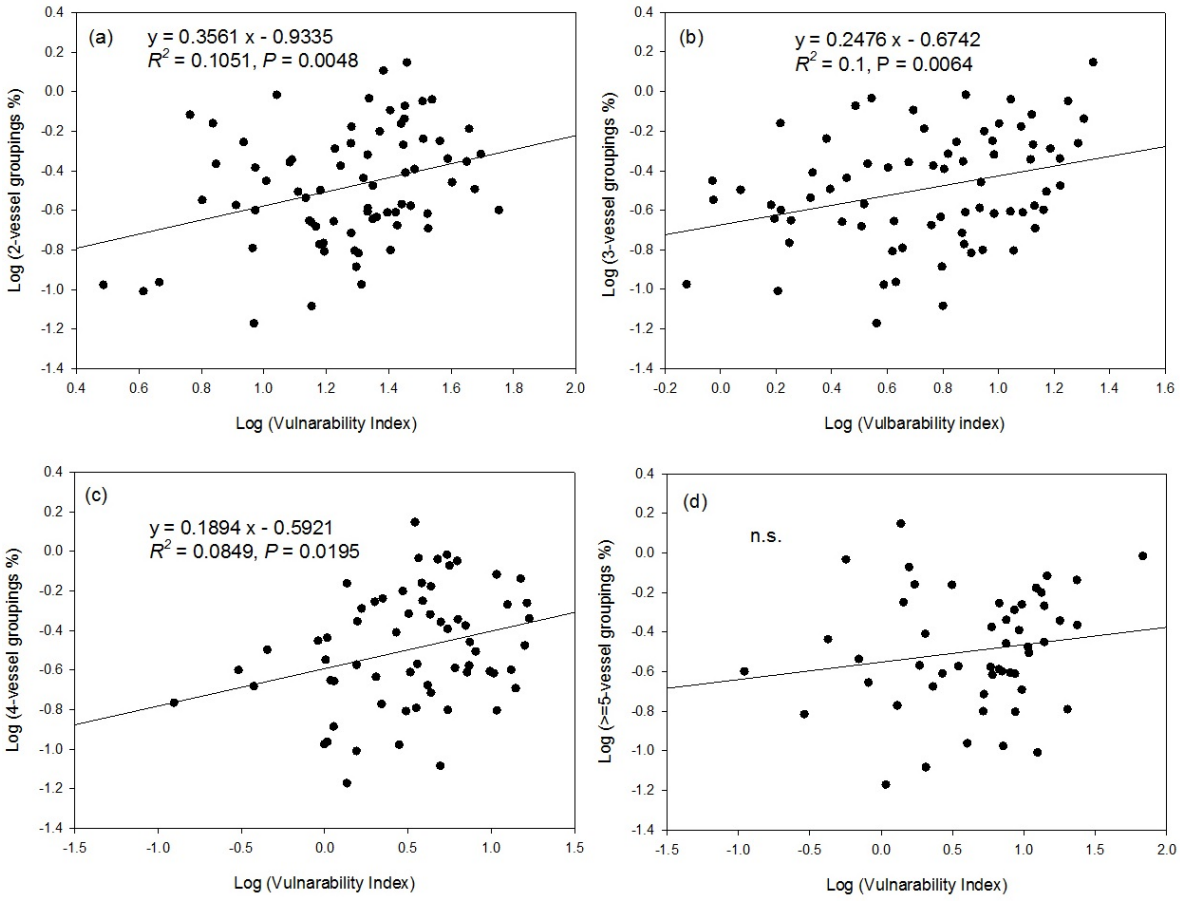

Figure 7 Relationship between vulnerability index and vessel grouping classifications. (a) the relationship with 2-vessel groupings; (b) 3-vessel groupings; (c) 4-vessel groupings; and (d) ≥ 5-vessel groupings.

Water use efficiency

Species represented 68.1% of the total variability in the model relative to the fixed factors, significantly contributing to differences in WUE ($P < 0.0001$). Light level was the only significant predictor of WUE, increasing 0.3 units per increase in light level ($P < 0.0001$). Origin had no effect on WUE. All four light levels were significantly different from each other; WUE increased as light levels increased ($P < 0.10$).

Discussion

Hydraulic contrasts in vessels between native and non-native species have been proposed in a number of recent studies (Pratt and Black 2006; Caplan and Yeakley 2010; Cavaleri and Sack 2010). However, we present results from the first study to examine the direct relationship between xylem vessel anatomical characteristics and water flow across such a large diversity of native and non-native woody shrub species. Contrary to our hypothesis, we found non-native woody species possessed smaller secondary xylem vessels compared with native species although the two groups had similar hydraulic conductance (Fig. 1, Fig. 2). Non-native, fast-growing species are often considered hydraulically efficient in that they exhibit xylem vessel characteristics that confer high water transport and reduced cavitation resistance (Markesteijn *et al*. 2011; Gilbert *et al*. 2006). The Hagan-Poiseuille law, which predicts that the hydraulic efficiency of a vessel increases with the fourth power of its diameter (Tyree and Zimmermann 2002), would suggest that fast-growing non-native species should have wider vessel diameter conduits, which would be advantageous for a rapid growth strategy.

No significant differences in WUE efficiency were found between native and non-native species ($P = 0.3973$). Water use efficiency (WUE) has been postulated to be

an important factor contributing to success of non-natives (Funk and Vitousek 2007), yet differences in WUE between non-native and native species is contingent upon growth form and climate (Cavaleri and Sack 2010). In co-occurring species of the same growth form, there is evidence to suggest that native and non-native species do not differ in water use efficiency, since WUE may act in conjunction with variations in other plant traits to weaken or remove competitive advantages (Cavaleri and Sack 2010; McAlpine *et al*. 2008; Funk and Vitousek 2007; Daehler 2003).

We found a significant positive correlation between xylem area as a percentage of wood area and hydraulic conductivity, which is consistent with Gleason *et al*. (2012) who found a positive correlation between xylem area and stem hydraulic conductivity across 120 Australian woody dicot species. However, there was not a relationship between conductivity and conduit traits of diameter and frequency, vulnerability index, and categorical porosity type (Fig. 2). Since vulnerability index was calculated directly from vessel diameter, it is unlikely that this measure would relate to K_s given the lack of relationship with vessel diameter. There was also no difference in conductivity between simple and scalariform plate types ($P > 0.10$), despite evidence from Christman and Sperry (2010) showing a considerable influence of vessel perforation plate type on xylem flow resistance in scalariform species. This finding may be due to the significant relatedness between individuals, since perforation plate type is conserved within groups of closely related species (Table 1). The relationship between hydraulic conductivity and resistance to cavitation events is well studied, with numerous findings of a trade-off between safety and efficiency (Pockman and Sperry 2000; Hacke *et al*. 2006; Sperry *et al*. 2008; Markesteijn *et al*. 2011). The extent of this relationship is highly dependent

upon adaptation of the xylem vessels (Markesteijn *et al*. 2011). A lack of difference in hydraulic conductance and vulnerability index in our study suggests that non-native plants do not exhibit a hydraulic advantage over native plants, a similar finding to that of Pratt and Black (2006). However, our lack of difference in hydraulic conductance and vulnerability index may be attributed to other anatomical factors such as perforation pits, which may account for > 50% of total flow resistance of vessel networks depending on number, size, and structure (Wheeler *et al*. 2005; Choat *et al*. 2008; Lens *et al*. 2010) and conduit length which is correlated with porosity type and conductivity per xylem cross-sectional area (Zimmermann and Jeje 1981; Lens *et al*. 2010). Additionally, our lack of difference may be due to the phylogenetic relationships among the species tested. Recent findings from Markesteijn *et al*. (2011) indicate that species differences can account for 62-98% of the variation in vessel traits. In our study, phylogenetic relatedness accounted for 39-68% of the total variation among traits. Comparing phylogenetic relatedness of native and non-native plants provided more meaningful explanation of invasive traits (McDowell 2002; Dawson *et al*. 2010). However, phylogenetic relatedness may constrain morphological and physiological variations between species (Goldberg 1987), which may explain the lack of difference in vessel traits observed in our study.

Non-native species had much higher (≥5) vessel groupings in metaxylem than native species. Metaxylem most likely becomes nonfunctioning after secondary xylem has developed, but it serves an important function during initial growth as in our species with only one year of growth. It is likely that the formation of metaxylem and vessel connectivity play an important role in the high growth rate of many non-native species. Maximum hydraulic conductivity has been found to increase with vessel connectivity

(Loepfe *et al*. 2007). Meanwhile, higher vessel groupings may also increase the vulnerability to cavitation by increasing the probability for the spread of embolism (Loepfe *et al*. 2007). In contrast to Lopfe *et al*. (2007), Carlquist (2009) and Lens *et al*. (2010) stated that vessel grouping would decrease the vulnerability to cavitation since it serves to bypass frequent embolisms by providing alternative routes for water flow. In our study, higher vessel groupings, especially the 2-, 3-, and 4-vessel groupings, showed increasing vulnerability to embolism, supporting the Loepfe *et al*. (2007) model for the potential of vessel connectivity to promote embolism. Interestingly, non-native species also had a higher proportion of small solitary vessels within their secondary xylem, which could partially explain the lack of difference in K_s between native and non-native species.

Average xylem vessel size was significantly smaller in non-native woody shrub species than in native species. In northern temperate deciduous forests, resistance to cavitation is an important feature in freeze tolerance. Davis *et al*. (1999) suggested a strong correlation between vessel diameter and cavitation by freezing, where small-vessel conduits are relatively resistant to cavitation. Thus, the smaller vessel diameter in non-native species may increase the competitive advantage of non-native species by allowing a longer growing season. In fact, recent work by Fridley (2012) has shown that non-native deciduous plants retain leaves longer through the autumn season than related native species thus allowing prolonged growth.

While our study incorporates a broad range of species, our scope is limited to stem hydraulic conductance, which might account for a small fraction of the total hydraulic conductance of a plant from roots to leaves (Tyree and Ewers 1991; Becker *et al*. 1998). Root mean vessel diameters were on average 30% larger than twig vessels in a

recent study of tropical trees by Schuldt *et al*. (2013), indicating an 85% increase in theoretical hydraulic conductance, as calculated by the Hagan-Poiseuille law. In future work, having a complete hydraulic architecture of non-native plants may better elucidate competitive mechanisms for water transport in non-native species. Moreover, a thorough evaluation of hydraulic conductance throughout an entire growing season is warranted as we suspect that larger differences in K_s may occur earlier in the spring when non-native species have functional metaxylem during shoot elongation.

Acknowledgements

We thank Meghan Horne for help with sectioning and embedding tissue samples, Vinay Pagay for running the HPFM and John Zhao for field help.

Works Cited

Baas P. 1983. Ecological patterns of xylem anatomy. *In* On the economy of plant form and function. Ed. Givinish T.J. Cambridge University Press, Cambridge, UK, pp. 327-351.

Caplan J.S. and Yeakley J.A. (2010) Water relations advantages for non-native *Rubus armeniacus* over two native ruderal congeners. *Plant Ecology* 210: 169-179.

Carlquist S. (1977) Ecological factors in wood evolution: a floristic approach. *American Journal of Botany* 64: 887-896.

Choat B., Cobb A.R., and Jansen S. (2008) Structure and function of bordered pits: new discoveries and impacts on whole-plant hydraulic function. *New Phytologist* 177: 608-625.

Cochard H. and Tyree M.T. (1990) Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiology*. 6: 393-407.

Dale M.P. and Causton D.R. (1992) The ecophysiology of *Veronica chamaedrys, V. Montana and V. officinalis*. II. The interaction of irradiance and water regime. *Journal of Ecology*. 80: 493-504.

Davis M.A., Grime J.P., and Thompson K. (2000) Fluctuating resources in plant communities: a general theory of invisibility. *Journal of Ecology*. 88: 528-534.

Drenovsky R.E., Khasanova A., and James J.J. (2012) Trait convergence and plasticity among native and invasive species in resource-poor environments. *American Journal of Botany*. 99: 629-639.

Fridley J.D. (2012) Extended leaf phenology and the autumn niche in deciduous forest invasions. *Nature*. 485 :359-364.

Funk J.L. and Vitousek P.M. (2007) Resource-use efficiency and plant invasion in low-resource systems. *Nature*. 446: 1079-1081.

Gilbert B., Wright S.J., Muller-Landau H.C., *et al*. (2006) Life history trade-offs in tropical trees and lianas. *Ecology*. 87: 1281-1288.

Goldberg D. (1987) Neighborhood competition in an old field plant community. *Ecology*. 68: 1211-1223.

Grotkopp E., Rejmanek M., and Rost T.L. (2002) Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. *American Naturalist*. 159: 396–419.

Hacke U.G., Sperry J.S., Pockman W.T., *et al*. (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia*. 126: 457-461.

Jansen S., Baas P., Gasson P., *et al*. (2004) Variation in xylem structure from tropics to tundra: Evidence from vestured pits. *Proceedings of the National Academy of Sciences*. 101: 8330-8837.

Lens F., Sperry J.S., Christman M.A., *et al*. (2011) Testing hypotheses that link wood anatomy to cavitation resistance and hydraulic conductivity in the genus Acer. *New Phytologist*. 190: 709-723.

Loepfe L., Martinez-Vilalta J., Piñol J., and Mencuccini M. (2007) The relevance of xylem network structure for plant hydraulic efficiency and safety. *Journal of Theoretical Biology*. 247: 788-803.

Markesteijn L., Poorter L., Bongers F., *et al*. (2011) Hydraulics and life history of tropical dry forest tree species: coordination of species' drought and shade tolerance. *New Phytologist*. 191: 480-495.

McCulloh K., Sperry J.S., Lachenbruch B., *et al*. (2010) Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. *New Phytologist*. 186: 439-450.

McCully M.E., Huang C.X., and Ling L.E.C. (1998) Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist*. 138: 327-342.

McDowell S.C.L. (2002) Photosynthetic characteristics of invasive and non-invasive species of *Rubus* (Rosaceae). *American Journal of Botany*. 89: 1431-1438.

Meinzer F.C., Clearwater M.J., and Goldstein G. (2001) Water transport in trees: current perspectives, new insights and some controversies. *Environmental and Experimental Botany*. 45: 239-262.

Pockman W.T. and Sperry J.S. (1996) Freezing-induced xylem cavitation and the northern limit of *Larrea tridentata*. *Oecologia*. 109: 19-27.

Pratt R.B. and Black R.A. (2006) Do invasive trees have a hydraulic advantage over native trees? *Biological Invasions*. 8: 1331-1341.

Sperry J.S., Nichols K.L., Sullivan J.E.M., and Eastlack S.E. (1994) Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of Northern Utah and interior Alaska. *Ecology*. 75: 1736-1752.

Steppe K. and Lemeur R. (2007) Effects or ring-porous and diffuse-porous stem wood anatomy on the hydraulic parameters used in water flow and storage model. *Tree Physiology*. 27: 43-52.

Taneda H. and Sperry J.S. (2008) A case-study of water transport in co-occurring ring-versus diffuse –porous trees: contrasts in water-status, conducting capacity, cavitation and vessel refilling. *Tree Physiology*. 28: 1641-1651.

Tyree M.T. and Ewers F.W. (1991) The hydraulic architecture of trees and other woody plants. *New Phytologist*. 119: 345-360.

Tyree M.T., Davis, S.D., and Cochard, H. (1994) Biophysical perspectives of xylem evolution – is there a trade-off of hydraulic efficiency for vulnerability to dysfunction. *Iawa Journal*. 15: 335-360.

Tyree M.T. and Zimmermann M.H. (2002) *Xylem Structure and the Ascent of Sap*. Berlin, Germany, Springer Verlag.

Conclusion:

Plant functional traits, measurable morphological and physiological characteristics responsible for plant performance, play a crucial role in the establishment, spread, and success of introduced non-native woody plants (Pysek and Richardson 2007; van Kleunen *et al*. 2010). Distinguishing differences in above and belowground traits between natives and non-natives is important to elucidating how non-native species may outcompete and displace natives, and potentially alter ecosystem processes and community structure. Here in two studies I compared both above and belowground traits of native and non-native woody understory species from a phenological perspective.

In the first study, I illustrated that two non-native species have the fine root capacity to co-opt soil resources earlier in the growing season, through differential timing in fine root growth and physiological nitrogen uptake. The high turnover and N uptake traits of non-native plant roots suggests similar belowground strategies that have been documented in a variety of other deciduous non-native woody species that exhibit high leaf production and carbon assimilation of leaves (Tecco *et al*. 2013). Additionally, Wolkovich *et al*. (2013) showed that non-native species growth in mesic temperate habitats might be tied with the ability of these species to closely track and respond to inter-annual differences in air temperature through phenological shifts compared to native species. Potentially, non-native plants may gain additional advantages through shifts in corresponding belowground root growth timing, since the on-set of the growing season coincides with fine-root growth (Steineker and Wilson 2008). The second study, which explored hydraulic properties among 83 native and non-native woody plants, revealed non-native species possessed aboveground xylem anatomical characteristics of wood that

support the potential for high early season water transportation and late season resistance to freeze-induced cavitation. Non-native species may gain advantages against native species through the potential to move water quickly during the spring period when phenology patterns shift due to inter-annual changes in air temperature, as well as extending growth into the fall through resistance to late season temperature fluctuations. Ideally, traits related to higher spring resource capture (e.g. early leaf out and root growth) and late-season tolerance to frost (e.g. small vessel diameters) is beneficial to non-native plants exhibiting extended growing seasons.

The ability for non-native plants to become invasive is complex, and is a reflection of the interaction of functional traits with abiotic conditions and biotic interactions (Brym *et al*. 2011, Huago *et al*. 2012, Dreiss and Volin 2013). Variation in plant traits may reflect trade-offs between tissue construction costs and function such as carbon fixation in leaves (Wright *et al*. 2004), root lifespan, root uptake (McCormack *et al*. 2012), and herbivore resistance and tolerance (Leger and Forister 2005, Ashton and Lerdau 2008). Likewise, trade-offs exist between mechanical properties of wood that permit hydraulic conductance and the safety of vessels from cavitation due to drought and freeze stress (reviewed in Lens *et al*. 2013). Understanding the role of functional traits and relationships with environmental factors could predict the invasive potential of non-native plants across different habitats.

Future work stemming from these two studies should continue to look deeper into the differences in functional traits between native and non-native woody plants. Fine root traits have rarely been explored in the context of invasive potential, and few studies have examined how environmental factors influence root lifespan (Comas and Eissenstat

2004). As globalization and climate change facilitate the spread of non-native plants, exploring the relationship of fine roots with manipulated conditions could provide insight into how native and non-native plants cope with environmental stress. For instance, while we did not find a hydraulic advantage in aboveground stems, how might hydraulic architecture of root systems support capabilities for water movement and distribution during periods of temperature change or water stress? Additionaly mycorrhizal association influences both water and nutrient absorption of fine roots as well as fine root lifespan is (Hodge 2009). Many invasive plants form myccorhizal associations, which can prolong root lifespan through potential pathogen resistance and enhanced nutrient absorption (King *et al*. 2002; Hodge 2009). Investigating mycorrhizal interactions of fine roots would further elucidate how non-native plants compete for belowground resources. Lastly, both herbivores and pathogens strongly impact plant performance and success. While much of the literature focuses on the release from herbivore pressure in new environments (Liu *et al*. 2007; Funk and Throop 2010), tolerance to herbivory may be an important strategy in many fast-growing plants that may invest less into defense (Coley 1988; Ashton and Lerdau 2008). Establishing a study to examine how non-native plants respond to both root and foliage herbivory could enhance the understanding of non-native plant persistence against damages.

Works Cited:

Ashton I.W. and Lerdau M.T. (2008) Tolerance to herbivory, and not resistance, may explain the differential success of invasive, naturalized, and native North American temperate vines. *Diversity and Distributions*. 14: 169-178.

Brym Z.T., Lake J.K., Allen D., and Ostling A. (2011) Plant functional traits suggest novel ecological strategy for an invasive shrub in an understory woody plant community. *Journal of Applied Eoclogy*. 48: 1098-1106.

Coley P.D. (1988) Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia*, 74: 531–536.

Comas L.H. and Eissenstat D.M. (2004) Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Functional Ecology*. 18: 388-397.

Dreiss L.M. and Volin J.C. (2013) Influence of leaf phenology and site nitrogen on invasive species establishment in temperate deciduous forest understories. *Forest Ecology and Management*. 296: 1-8.

Funk J.L. and Throop H.L. (2010) Enemy release and plant invasion: patterns of defensive traits and leaf damage in Hawaii. *Oecologia*. 162: 815-823.

Hodge A. (2009) Plant root growth, architecture and function. *Plant and Soil*. 321: 153-187.

Huago R.D., Bakker J.D., and Halpern C.B. (2013) Role of biotic interactions in regulating conifer invasion of grasslands. *Forest Ecology and Management*. 289: 175-182.

King J.S., Albaugh T.J., Allen H.L., *et al*. (2002) Below-ground carbon input to soil is

controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist*. 154: 389-398.

Leger E.A. and Forister M.L. (2005) Increased resistance to generalist herbivores in invasive populations of the California poppy (*Eschscholzia californica*). *Diversity and Distributions*. 11: 311-317.

Lens F., Tixier A., Cochard H., *et al*. (2013) Embolism resistance as a key mechanism to understand plant adaptive strategies. *Current Opinion in Plant Biology*. 16: 287-282.

Liu H., Stilling P., and Pemberton R.W. (2007) Does enemy release matter for invasive plants? Evidence from a comparison of insect herbivore damage among invasive, non-invasive and native congeners. *Biological Invasions*. 11: 773-781.

McCormack M.L., Adams T.S., Smithwick E.A.H., and Eissenstat D.M. (2012) Predicting fine root lifespan from plant functional traits in temperate trees. *New Phytologist.* 195: 823-831.

Pysek P. and Richardson D.M. (2007) Traits associated with invasiveness of alien plants: Where do we stand? *Biological Invasions*. 193: 97-125.

Steinaker D.F. and Wilson S.D. (2008) Phenology of fine roots and leaves in forest and grassland. *Journal of Ecology*. 96: 1222-1229.

Tecco P.A., Urcelay C., Diaz S., *et al*. (2013) Contrasting functional trait syndromes underlay woody alien success in the same ecosystem. *Austral Ecology*. 38: 443-451.

Wolkovich E.M., Davies T.J., Schaefer H., *et al*. (2013) Temperature-dependent shifts

in phenology contribute to the success of exotic species with climate change. *American Journal of Botany*. 100: 1407-1421.

van Kleunen M., Weber E., and Fischer M. (2010) A meta-analysis of trait differences between invasive and non-invasive plants. *Ecology Letters*. 13: 235-245.

Wright I.J., Reich P.B., Westoby M., *et al*. (2004) The world-wide leaf economics spectrum. *Nature*. 428: 821-827.